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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : B01J 19/00 | A2

(11) International Publication Number: WO 00/18503

(43) International Publication Date: 6 April 2000 (06.04.00)

(21) International Application Number: PCT/GB99/03200

(22) International Filing Date: 24 September 1999 (24.09.99)

(30) Priority Data:
9820893.7 26 September 1998 (26.09.98) GB

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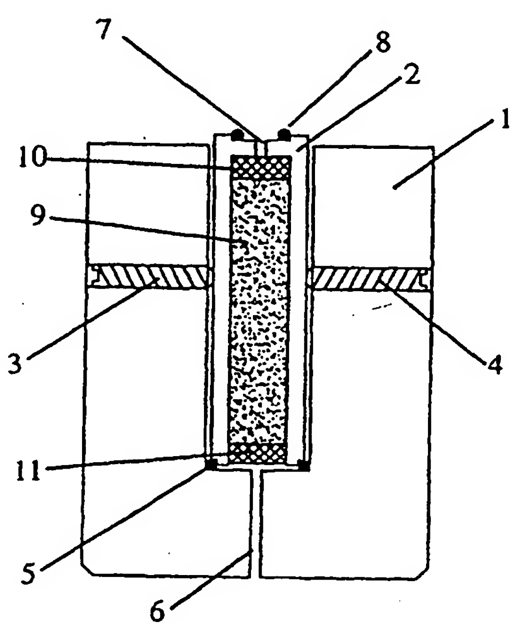
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: COMBINATORIAL SYNTHESISER

(57) Abstract

Equipment for performing automated combinatorial chemical synthesis of oligonuclestidy and peptides has a number of reaction capsules each with a pressure plate operable by actuators to permit fluid delivery and fluid collection. The capsules are moved between stations in the equipment, synthetic operations are performed, synthesis products are tested, capsules are removed and replaced, synthetic conditions are altered if necessary, and the synthetic operations are reiterated if necessary, all as appropriate.



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COMBINATORIAL SYNTHESISER

This invention relates to apparatus and methods for performing combinatorial chemical syntheses.

Combinatorial methods have been employed in a number of different fields of synthetic organic chemistry. In general these methods involve contacting a solid-support material with one or more reactant fluids in order to produce a desired product. This solid-phase synthesis technique was originally applied to the synthesis of peptides by Merrifield, J. Amer. Chem. Soc., 1963, vol. 85, pages 2149-2154 and subsequently to the synthesis of oligonucleotides, as described, for example, by Letsinger et al., Nucleic Acids Research, 1975, vol. 2, pages 773-786.

More recently, these techniques have been applied in other areas, for example to produce chemical libraries of benzodiazepines, as described by Ellman in U.S. Patent No. 5,288,514.

A primary requirement in generating such combinatorial libraries is the ability to perform large numbers of syntheses in parallel, and apparatus for achieving such multiple parallel syntheses has been described in several patent documents, for example by Urdea in EP0130739, by Niina in US4671941, by Hamill in EP0164206, by Judd in US5053454, by Ansorge in EP0365668, by Zuckermann in EP0593460, by Church in EP0586600, by McGraw in EP0683790, by Chang in US5380495, by Nokihara in EP0529504, by Sugarman in WO9512608, by Brennan in EP0734397, by Delius in US5538694, by Scott in WO9817391 and by Moore in WO9745455.

Although such apparatus is efficient in the synthesis of oligonucleotides and peptides, attempts to extend the use of these automated systems to other fields of synthetic organic chemistry have proved less successful. In particular, there exists a need for apparatus which is capable of optimising the reaction conditions employed to synthesise a series of compounds. In order to achieve this, it is necessary to devise an apparatus which is capable of initiating new syntheses automatically so that the apparatus is capable of continuous unattended operation.

This presents a particular difficulty in the field of solid-phase synthesis, since the solid-support used for each synthesis is commonly contained in a reaction column which is fitted to the instrument manually. A very commonly used configuration in oligonucleotide synthesis uses a cartridge with tapered Luer connectors at either end, which "plugs in" to the instrument before commencing synthesis. A variation of this type of column with modified end-fittings to simplify post-synthesis handling has been described by Coassin in U.S. Patent No. 5,419,874. However, neither of these types of column is suitable for automated handling, for example in a robotic system.

The present invention provides a method as defined in Claim 1 hereinafter.

The method may include the features of any one or more of dependent Claims 2 to 15.

The present invention also provides a computer program as defined in Claim 17 hereinafter.

The present invention also provides a reaction capsule as defined in Claim 19 hereinafter.

The reaction capsule may include the features of any one or more of dependent Claims 20 to 27.

The present invention also provides equipment as defined in Claim 28 hereinafter.

The equipment may include the features of any one or more of dependent Claims 29 to 31.

I have now found that the problems above may be overcome by use of a suitable apparatus which, advantageously, allows unattended synthesis of large numbers of compounds, is capable of automatically optimising reaction conditions, and is economical and rapid in operation.

According to the present invention, there is provided a reaction capsule which incorporates a reaction zone in which a solid-support may be contained, retention means for retaining the solid-support in the reaction zone, inlet and outlet conduits for the passage of fluids through the reaction zone, optionally, valve means in the inlet and outlet conduits to allow fluid to be retained in the capsule, and, optionally, window means through which the contents of the capsule may be observed or analysed by spectrophotometric methods, said reaction capsule being adapted to form a first fluid-tight seal between the inlet conduit and a complementary fluid-delivery port and a second fluid-tight seal between the outlet conduit and a complementary fluid-collection port, both said ports being external to the capsule, characterised in that

(i) the said fluid-tight seals are activated solely by the application of pressure between said capsule and said external ports, and

(ii) the said fluid-tight seals are deactivated in the absence of applied pressure between said capsule and said external ports.

The present invention further provides an apparatus which comprises:

(i) a multiplicity of movable reaction capsules, each equipped with identification means, and each of which incorporates a reaction zone in which a solid-support may be contained, retention means for retaining the solid-support in the reaction zone, inlet and outlet conduits for the passage of fluids through the capsule, optionally, valve means in the inlet and outlet conduits to allow fluid to be retained in the capsule, and, optionally, window means through which the contents of the capsule may be observed or analysed by spectrophotometric methods

and

(ii) a multiplicity of fluid-delivery and fluid-collection ports complementary to the inlet and outlet conduits of the reaction capsules, the fluid-delivery ports being connected to valve and delivery means to allow selection of fluids to be delivered to each capsule, and the fluid-collection ports being connected to collection means to allow fluids emerging from each capsule to be collected,

and

(iii) sealing means to allow passage of fluid through the capsules without leakage

and

(iv) movement means allowing each capsule to be brought into contact with any pair of fluid-delivery and fluid-collection ports,

and

(v) releasable compression means allowing each capsule to form a fluid-tight seal with the complementary fluid-delivery and fluid-collection ports, while allowing each capsule to be released as required in order to allow movement of the capsule,

and

(vi) replacement means allowing each capsule to be removed from the apparatus and replaced with a different capsule,

and, optionally,

(vii) monitoring means allowing the progress of chemical processes taking place within each reaction capsule to be monitored,

and, optionally,

(viii) temperature-control means allowing the temperature of one or more reaction capsules to be varied.

The invention yet further provides a method for performing a multiplicity of combinatorial chemical syntheses which comprises providing apparatus as aforesaid, in which the solid-supports contained in each capsule and the fluids available for delivery to the delivery ports are chosen so as to be appropriate to the syntheses to be performed, moving each capsule to an appropriate position according to the compound to be synthesised in that capsule, activating the compression means to create a fluid-tight seal between the delivery and collection ports and the inlet and outlet conduits of each capsule, delivering fluids to each capsule appropriate to the compound to be synthesised in that capsule, optionally monitoring the progress of the chemical processes taking place in each capsule, deactivating the compression means to allow each capsule to be moved to a new position, optionally removing any capsules in which all necessary synthesis operations have been completed and replacing them with capsules in which further synthetic operations are to be performed, and repeating the same sequence of operations as often as required in order to complete all necessary synthetic

operations to achieve synthesis of the desired products.

The invention yet further provides a method for optimising reaction conditions for combinatorial chemical syntheses which comprises applying the method as aforesaid, monitoring the progress of reaction in each capsule for each step of the synthesis, optionally removing one or more capsules and replacing them with new capsules and repeating the previous sequence of synthetic operations after optionally making alterations to the quantity, rate of delivery or identity of fluids delivered to each capsule during each synthetic operation, optionally altering the time allowed for completion of each synthetic operation in each capsule, and optionally varying the temperature of each capsule during each synthetic operation, all of the above steps then being reiterated as often as required until no further improvement in progress of reaction is observed.

Specific embodiments of the invention will now be described, by way of example, with reference to the accompanying drawings, in which:-

Figure 1 shows a reaction capsule in cross-section.
Figure 2 shows a reaction capsule in side view.
Figure 3 shows a reaction capsule in top view.
Figure 4 shows a reaction capsule in bottom view.
Figure 5 shows the inner part of a reaction capsule in cross-section.
Figure 6 shows the inner part of a reaction capsule in perspective view.
Figure 7 shows diagrammatically, in perspective view, a reaction capsule together with the complementary fluid-delivery and fluid-collection ports.
Figure 8 shows, in cross-section, a reaction capsule together with the complementary fluid-delivery and fluid-collection ports..
Figure 9 shows, in cross-section, a reaction capsule with internal spring-operated ball valves in the inlet and outlet conduits.
Figure 10 shows, in cross-section, a reaction capsule with internal magnetically-operated ball valves in the inlet and outlet conduits.
Figure 11 shows, in perspective view, a reaction capsule with windows in the side-walls.

Figure 12 shows, in cross-section, the inner part of a reaction capsule with transparent side-walls.

Figure 13 shows, in cross-section, the inner part of a reaction capsule with transparent side-walls, with an insert for collecting solid-support material for analysis.

Figure 14 shows, in cross-section, the inner part of a reaction capsule with windows in the side-walls.

Figure 15 shows, in cross-section, a reaction capsule with inlet and outlet conduits on the same face of the capsule, together with the complementary fluid-delivery and fluid-collection ports, and a heating/cooling block.

Figure 16 shows, in top-view, a reaction capsule with inlet and outlet conduits on the same face of the capsule.

Figure 17 shows, in cross-section, a flanged reaction capsule with inlet and outlet conduits on the same face of the capsule, together with the complementary fluid-delivery and fluid-collection ports and a retaining mechanism.

Figure 18 shows, in cross-section, an apparatus containing multiple reaction capsules together with the complementary fluid-delivery and fluid-collection ports.

Figure 19 shows, in side-view, an apparatus containing multiple reaction capsules together with the complementary fluid-delivery and fluid-collection ports and a compression mechanism in the compressed state.

Figure 20 shows, in side-view, an apparatus containing multiple reaction capsules together with the complementary fluid-delivery and fluid-collection ports and a compression mechanism in the uncompressed state.

Figure 21 shows, in end-view, an apparatus containing multiple reaction capsules together with the complementary fluid-delivery and fluid-collection ports and a compression mechanism in the uncompressed state.

Figure 22 shows, in top-view, an apparatus containing multiple reaction capsules together with a movement mechanism.

Figures 23-31 show, in plan view, the position of capsules in the apparatus after each step in the operation of the movement mechanism.

Figure 32 shows, in perspective view, a capsule with identification means.

Figure 33 shows, in plan view, an apparatus incorporating a light source and

detector.

Figure 34 shows diagrammatically the plumbing layout of the apparatus.

Figure 35 shows schematically a combinatorial system containing two instruments.

Figure 36 shows schematically a combinatorial system containing three instruments.

Referring to the drawings, in the embodiment illustrated in Figures 1-6 inclusive, the reaction capsule consists of two parts: an outer cuboidal part 1 and an inner cylindrical part 2, the top end of which protrudes slightly above the top surface of the outer part 1. The inner part 2 is secured by retaining screws 3 and 4 and an O-ring 5 at the base of the inner part forms a fluid-tight joint with the inlet conduit 6 of the outer part 1, the direction of fluid flow being upwards through the capsule. The opposite end of the inner part has an outlet conduit 7 and a further O-ring 8 which is retained in a recess round the conduit 7. The inner part contains an interior cylindrical chamber which may contain a solid-support material 9, retained by porous filters 10 and 11.

The capsule together with the complementary fluid-delivery and fluid-collection ports is further illustrated in Figures 7 and 8, in which the fluid-delivery port 12 which delivers fluid to the base of the capsule contains a central fluid passage 13 with an O-ring 14 retained in a recess round the passage 13 which is attached by means of a connector 15 to tubing 16. The fluid-collection port 17, which collects fluid from the top of the capsule is similarly attached by means of connector 18 to tubing 19 which communicates with central fluid-passage 20. The fluid-delivery port 12 is mounted in a recessed hole 21 in a base-plate 22 and is held in place by lock-nut 23 which engages on the outer threaded portion 24 of the body of the fluid-delivery port 13, such that the top surface of the port with the O-ring 14 protrudes slightly above the top surface of the base plate 22. The fluid-collection port 17 is mounted in a hole 25 in a pressure-plate 26 and is held in place by lock-nut 27 which engages on the outer threaded portion 28 of the body of the fluid-delivery port 17. The pressure plate also contains springs 29 and 30 mounted in recesses 31 and 32, which bear down on the lower part of the fluid-collection port 17 which seals against the O-ring 8 of the reaction capsule. This arrangement compensates for slight misalignments of the capsule and also allows for variations

in the height of the capsules due to manufacturing tolerances.

Preferred materials for the construction of the capsule components 1 and 2 and the fluid-delivery and fluid-collection ports 12 and 17 are chemically-resistant plastics such as PEEK, PTFE or polypropylene or ceramic materials such as MACOR®. Preferred materials for the O-ring seals 5, 8 and 14 are fluorocarbon rubber, Viton® or EPDM. Preferred materials for the base-plate 22 and pressure-plate 26 are chemically-resistant plastics such as PEEK or Acetal. Preferred materials for the filters 10 and 11 are porous PTFE or porous polypropylene. Preferred materials for the lock-nuts 23 and 27 are polypropylene or Tefzel®. A preferred material for the screws 3 and 4 and the springs 29 and 30 is stainless steel. The preferred material for the tubes 16 and 19 is PTFE.

In the embodiment illustrated in figure 9, the inner part 2 of the capsule additionally contains valves in the inlet and outlet passages on either side of the filters 10 and 11 and solid-support bed 9. These valves consist of housings 33 and 34 providing valve seats for the balls 35 and 36 which are held in place by springs 37 and 38. The springs are selected so as to allow the valves to close and fluid to be retained in the capsule in the absence of applied fluid pressure, but to open when fluid under pressure is delivered to the capsule, allowing flow through the capsule. Preferred materials for the housings 33 and 34 are PEEK, PTFE or MACOR®. Preferred materials for the balls 35 and 36 are borosilicate glass, PEEK, PTFE or MACOR®. Preferred materials for the springs 37 and 38 are stainless steel or titanium.

A further embodiment illustrated in figure 10 also contains ball valves in the inlet and outlet conduits which are held closed by magnetic force but may be opened by application of an external magnetic field. In this configuration, the housings 39 and 40 again provide seating for the balls. These are of two-part construction, comprising outer spherical parts 41 and 42 which may be made of chemically inert materials such as PTFE or polypropylene enclosing inner spheres 43 and 44 which are made of ferromagnetic material and are held in place against the valve seats by the attraction of magnets 45, 46, 47 and 48 embedded in recesses in the outer part of the capsule. This arrangement allows the opening of valves to be controlled by

application of an external magnetic field which is capable of counteracting the attraction of the magnets fitted to the capsule itself, and further allows construction of the capsule without exposing metallic components to the reactant fluids.

A further embodiment illustrated in figures 11 - 14 inclusive shows the construction of a capsule with windows for observation or spectrophotometric analysis of the contents of the capsule. The outer part of the capsule contains an aperture 49 on one face and a similar aperture on the opposing face, allowing passage of a light beam through the inner part of the capsule. The inner part is constructed of end-fittings 50 and 51 with fluid passages 52 and 53 and O-rings 54 and 55, the end-fittings being attached to a transparent tube 56 which has filters 10 and 11 fitted to either end and is partially filled with solid-support material 9. This arrangement allows a supernatant liquid above the solid-support to be analysed by spectrophotometric methods. Alternatively, the tube 56 may contain an insert 57 which carries a sampling disc 58. The purpose of the sampling disc is to trap a small sample of the solid support material for analysis, for example by Infra-Red spectrophotometry. This may be achieved by tilting the capsule to a horizontal position and passing a stream of gas through the capsule at a rate sufficient to carry particles of the solid-support material along the insert to the sampling disc.

A third configuration of capsule employs an inner cylindrical section 59 with an O-ring 60 and flow passage 61 on the top surface, an O-ring 62 at the base, and windows 63 and 64 fitted in the side walls of the tube, which is fitted with filters 10 and 11 at either end and is partially filled with solid-support material 9. This arrangement allows both a supernatant liquid and the solid-support to be examined as required.

Preferred materials for the construction of the end-fittings 50 and 51, the insert 57 and the tube 59 are PEEK, PTFE or polypropylene. Preferred materials for the transparent tube 56, and the windows 63 and 64 are dependent on the wavelength at which the spectrophotometric analysis is to be performed and may include glass, fused silica or alumina, silicon carbide, calcium fluoride and potassium bromide. The sampling disc 58 may likewise be constructed from these or similar materials, preferably in a sintered form.

A further embodiment of the capsule of the invention is illustrated in Figures 15 and 16. In this embodiment, the inner cylindrical section of the capsule is made up of an upper section 65 and a lower section 66 which are held together by a screw thread and are retained in the outer part of the capsule 67 by retaining screws 68 and 69 and contain an internal O-ring seal 70 . The top surface of the upper section 65 has an outlet conduit 74 separated by an O-ring 75 from an inlet conduit 76 which is in turn surrounded by a further O-ring 77. The inlet conduit 76 connects with an annular internal channel 79 which in turn connects with a further flow-passage 78 leading to the internal chamber in which filters 72 and 73 retain the solid-support bed 71.

The complementary fluid-delivery and fluid-collection ports are incorporated in a single component 80 mounted on a pressure plate 81 and retained by a lock-nut 82 which engages with the threaded section 83 of the component. Springs 84 and 86 are held in recesses 85 and 87 in the pressure-plate and bear against the sealing surfaces to allow for manufacturing tolerances in the capsule and compensate for minor misalignment. The fluid-delivery conduit 88 is engaged through connector 89 to supply tube 90 and the fluid-collection conduit 91 is engaged through connector 92 to drain tube 93. It should be noted that precise alignment of the inlet tube 76 of the capsule with the fluid-passage 88 of the connector is not required, since fluid is able to flow freely round the annular space between the O-rings 75 and 77.

Since there are no fluid connections to the base of the capsule in this embodiment, a simple base plate may be employed. Alternatively, as illustrated in Figure 15, a composite base plate made up of a Peltier-effect thermal control device 94 and a heating/cooling block 95 may be used. This allows the reaction capsule to be heated or cooled as required in order to optimise synthesis conditions. In this case, preferred materials for the outer cuboidal section 67 of the capsule are aluminium, copper, bronze or stainless steel. Where heating and cooling are not required, materials such as PEEK, polypropylene or acetal are preferred for this component. Preferred materials for the construction of the inner capsule components 65 and 66 and the fluid-delivery and fluid-collection component 80 are PEEK, PTFE, polypropylene or ceramic materials such as MACOR . Preferred materials for the O-ring seals 70, 75 and 77 are fluorocarbon rubber, Viton® or EPDM. Preferred

materials for the pressure-plate 81 are chemically-resistant plastics such as PEEK or Acetal. Preferred materials for the filters 72 and 73 are porous PTFE or porous polypropylene. Preferred materials for the lock-nut 82 are polypropylene or Tefzel®. A preferred material for the screws 68 and 69 and the springs 84 and 86 is stainless steel. The preferred material for the tubes 90 and 93 is PTFE.

A further embodiment of the capsule of the invention is illustrated in figure 17. As in the previously described embodiment, the inner cylindrical section of the capsule is made up of two components 65 and 66 and has both inlet and outlet conduits on its top surface. In this case, however the outer section of the capsule is fitted with flanges 96 and 97 which engage with retaining clips 98 and 99 mounted by means of pivots 100 and 101 on the pressure-plate 103 so as to seal against the port connector 80. The clips 98 and 99 are held in position by springs 108 and 109 mounted on pins 106 and 107 held in brackets 104 and 105 which are mounted on the pressure-plate. The clips 98 and 99 may be released by actuators 110 and 111 operating through push-rods 112 and 113, allowing the capsule to be removed, for example by a robotic arm. The actuators 110 and 111 may be pneumatic cylinders or solenoids. In this embodiment, the external form of the outer part of the capsule may be cuboidal or cylindrical. Preferred materials for construction of the retaining clips 98 and 99 are polypropylene or acetal. Preferred materials for other components are as detailed in the previous embodiments.

Embodiments of the invention incorporating multiple reaction capsules will now be described. For the purposes of this description, the first of the previously-described embodiments of the reaction capsule will be employed as the exemplary form of capsule. However, it will be understood that the additional features of valves, windows and configuration of inlet and outlet conduits described in the other previously-described embodiments are also intended to be applied in an apparatus incorporating multiple reaction capsules and such apparatus forms part of the invention irrespective of the type of reaction capsule employed.

An embodiment of the invention incorporating multiple reaction capsules is illustrated in cross-sectional view in Figure 18, which shows four pairs of fluid-delivery and fluid-collection ports mounted on the same base-plate 114 and pressure-plate 115, together with four reaction capsules. The base-plate extends beyond the rightmost of these capsules, whereas the pressure-plate covers only the

immediate area of the capsules. The side view of the apparatus is shown in Figure 19, which also shows the pneumatic cylinder 117 which connects to the pressure-plate 115 through a push-rod 116, applying pressure to the pressure-plate 115 to compress the capsules against the base-plate 114.

When the pneumatic cylinder 117 is retracted, the pressure-plate is raised, releasing the reaction capsules. This is illustrated in Figure 20, which shows a side-view of the apparatus when the capsules are uncompressed. The end-view of the apparatus in this condition is shown in Figure 21, from which it can be seen that there are two rows of four capsules in the apparatus. This view also shows the guide rods 118 and 119 which maintain the alignment of the pressure-plate with the base-plate during raising and lowering of the pressure-plate. These rods are fixed to the base-plate and engage in holes in the pressure-plate allowing the pressure-plate to move freely up and down while maintaining alignment with the base-plate.

For clarity, these guide rods and the pressure plate and pneumatic cylinder 117 are omitted from subsequent drawings. These describe the layout of the capsules during operation of the apparatus.

Figure 22 shows in plan view the arrangement of the reaction capsules in the apparatus during operation. In this drawing the capsules are shown in the position which they occupy when the pressure-plate is engaged. In this configuration, the capsules are aligned with the fluid-delivery and fluid-collection ports and fluid can be delivered independently to each capsule and collected independently from each capsule through the supply and drain tubes. The two rows of four capsules are retained between guide rails 120, 121 and 122 which bear against the base of the capsules on three sides of the apparatus. Surrounding the capsules are six actuators. In the diagram these are shown as pneumatic cylinders, although electrical solenoids may also be employed. Actuator 1 may be extended by applying pressure from a compressed air supply through the tube 123 and retracted by applying pressure through tube 124. Similarly, Actuator 2 may be extended by applying pressure through tube 125 and retracted by applying pressure through tube 126, Actuator 3 may be extended by applying pressure through tube 127 and retracted by applying pressure through tube 128, Actuator 4 may be extended by

applying pressure through tube 129 and retracted by applying pressure through tube 130, Actuator 5 may be extended by applying pressure through tube 131 and retracted by applying pressure through tube 132, and Actuator 6 may be extended by applying pressure through tube 133 and retracted by applying pressure through tube 134. In the normal configuration of the apparatus, all the actuators are in the retracted state except for Actuator 5, which is normally in the extended state.

Actuators 1-4 inclusive are used to reposition the capsules. Actuator 5 operates a stop-bar which restricts the movement of the capsules during repositioning. This actuator is positioned below the level of Actuator 4 so that both actuators can be operated independently without the pistons colliding. Actuator 6, which has a longer operating stroke than the other actuators, carries a vacuum pad which may be connected to a vacuum source through tube 135 by operation of a solenoid valve. Further solenoid valves control the operation of the actuators, and these are in turn controlled by a computer which also controls the supply of fluids to the fluid-delivery ports through additional valves. For simplicity, these valves are omitted from the drawings.

Figure 22 shows the capsules in a particular configuration, indicated by the labels A,B,C,D,E,F,G and H on the capsules. In this position, after operation of the compression means to form a fluid-tight seal between the capsules and the fluid-delivery and collection ports, each capsule may be contacted with a range of reaction fluids in order to carry out a particular chemical process. When these processes have been completed, further chemical treatments may be required in each capsule, necessitating a move of each capsule to a different position. Alternatively, if all chemical processes in a capsule have been completed, it may be necessary to remove a capsule from the apparatus and replace it with a new capsule. In either case, the necessary movement of the capsules may be accomplished by operating the actuators appropriately. After releasing the compression means by raising the pressure-plate, the capsules are freely movable. The sequence of operations begins with the extension of actuator 1 to move capsules E,F,G and H. The stroke-length of each of the actuators 1 to 5 inclusive is selected or adjusted so that it is equal to the side dimension of the capsules. The configuration of capsules after the extension of actuator 1 is illustrated in Figure 23. The stop bar prevents further travel of the capsules in the direction of

movement, and movement on the remaining three sides of the base-plate is constrained by the guide rails.

The second step in the process of moving the capsules requires the retraction of actuator 1 and the subsequent extension of actuator 2, thus moving capsule D into the vacant space created by the previous operation. The configuration of the capsules after this step is illustrated in Figure 24.

The third step in the process of moving the capsules requires the extension of actuator 3, thus transferring capsule H into the adjacent row of capsules. The configuration of the capsules after this step is illustrated in Figure 25.

The fourth step in the process of moving the capsules requires the retraction of actuators 2 and 3 and the extension of actuator 4, thus moving capsules C,B,A and H back into alignment with the fluid-delivery and fluid-collection ports. The configuration of the capsules after this step is illustrated in Figure 26.

All eight capsules are now aligned with the fluid-delivery and fluid-collection ports in the base-plate and pressure-plate, so that, after operation of the compression means to form a fluid-tight seal between the capsules and the fluid-delivery and collection ports, each capsule may again be contacted with a range of reaction fluids. However, since each capsule is now aligned with a different pair of fluid-delivery and collection ports, as may be demonstrated by comparing the original positions of the capsules as shown in Figure 22, and the new positions of the capsules as shown in Figure 26, the range of fluids available for delivery to each capsule may also be different. Comparison of Figures 22 and 26 also demonstrates that, after retraction of actuator 4, all the actuators will also returned to their original positions. The sequence of operations described above therefore provides the capacity to perform cyclic chemical processes using the apparatus.

A further requirement of the apparatus is the ability to remove a reaction capsule on which all synthetic operations have been completed and to replace it with another reaction capsule to initiate a new series of synthetic operations. This may also be achieved using appropriate movements of the actuators. Referring to Figure 24, it will be noted that further movement of capsule H is prevented by the stop-bar connected to actuator 5, which, as previously described, is normally in the extended position. Retraction of actuator 5 when the capsules are in the configuration shown in Figure 24 produces the layout illustrated in Figure 27.

Subsequent extension of actuator 6 then produces the configuration illustrated in Figure 28. It should be noted that, as previously described, the stroke-length of actuator 6 is greater than that of the other actuators.

The next step required is the application of vacuum to the vacuum pad, followed by retraction of actuator 6. This shifts capsule H to a position clear of the overlying pressure-plate, as illustrated in Figure 29. In this position, after releasing the vacuum pad, the capsule may be removed, for example by use of a robotic arm, and replaced with a different capsule. This is illustrated in Figure 30, in which capsule H has been replaced by a new capsule, here labelled capsule X. This new capsule may be returned to the position formerly occupied by capsule H by extending actuator 6 without applying vacuum to the vacuum pad, retracting actuator 6, and extending actuator 5 to return the stop-bar to its normal position. The layout of the capsules after these operations is illustrated in Figure 31. Comparison of Figure 31 with Figure 24 confirms that the configuration of the capsules is identical except that capsule X has replaced capsule H. Completion of the previously described sequence of operations as illustrated in Figures 25 and 26 will therefore place the new capsule X in the positions occupied by capsule H in these figures, thus allowing fluids to be delivered to this new capsule.

The apparatus is designed to allow large numbers of syntheses to be carried out by removing capsules from the system when all synthetic operations have been completed in those capsules and replacing such capsules with further capsules in order to carry out further syntheses. Each capsule must therefore carry identification means, for example as shown in Figure 32, which illustrates a capsule carrying a bar-code label 136 and which also has a uniquely encoded microchip or RF-tag 137 embedded in its side-wall, by means of which the capsule may be identified using a suitable transponder or decoder. The identity of each capsule, whether obtained from a bar-code reader or from an RF decoder, may therefore be determined automatically by the control computer, which is thus able to keep track of all the capsules in the system.

The apparatus may include monitoring means, for example spectrophotometric means. Where capsules are fitted with windows, the monitoring means may be incorporated into the instrument. For example, Figure 33 shows the apparatus in the configuration previously illustrated in Figure 29, with the addition of a light-

source 138 and a detector 139. Alternatively, a flow-cell may be incorporated in one or more of the drain tubes carrying fluids away from the capsules so that these fluids may be monitored spectrophotometrically. The flow-cell may alternatively or additionally include other monitoring devices, for example electrodes to measure conductivity, capacitance, pH or ion-specific electrodes, or sensors to measure other properties of the fluid, for example temperature or turbidity.

Further, a capsule may be removed from the apparatus as previously described and transferred, for example using a robotic arm, to a separate apparatus which is adapted for spectrophotometric analysis. This arrangement is of particular utility where the solid-support material is to be analysed, for example by infra-red spectrophotometry. As described previously, a capsule may incorporate a sampling disc on which particles of solid-support may be trapped for such analytical procedures. In order to transfer such particles to the sampling disc, the capsule is tilted and a stream of gas passed through the capsule to mobilise the particles. This may be achieved by use of a robotic arm in conjunction with a gas delivery system. The robotic arm may also serve to position or hold the capsule in the beam of a spectrophotometer.

Other monitoring means may also be used to analyse the contents of capsules which have been removed from the apparatus, for example chromatographic means. In order to use such means, a capsule may be transferred, for example using a robotic arm, to a separate apparatus which is adapted to remove an aliquot from the contents of the capsule for transfer to a chromatographic analyser equipped with an automatic sample injector. The aliquot so removed may be either a sample of the liquid contents of the capsule, or, where the material bound to the solid-support is of interest, a liquid may be introduced to the capsule in order to liberate and solubilise part of the support-bound material from the support. The chromatographic means used may make use of gas chromatography, liquid chromatography, ion-exchange chromatography or other chromatographic techniques, and may be applied either alone or in conjunction with mass spectrometry. Other techniques such as electrophoresis or gel filtration may equally be applied to the analysis of such aliquot samples, as well as spectrophotometric techniques. These may include ultra-violet, infra-red and visible spectrophotometry, spectrofluorimetry, atomic absorption spectroscopy,

flame photometry, nuclear magnetic resonance spectroscopy and electron-spin resonance spectroscopy.

A particularly useful method of removing an aliquot of support-bound material for analysis may be employed in cases in which the capsule is equipped with windows as previously described and the support-bound material is linked to the solid-support by means of a photochemically-cleavable linking group. Such groups have been described, for example, by Holmes in U.S. Patent No. 5,679,773. In such cases, the capsule may be removed from the apparatus and transferred, for example using a robotic arm, to a separate apparatus which is adapted to remove an aliquot of support-bound material by irradiation of the solid-support at a suitable wavelength through the windows of the capsule. The material of the windows is selected for high transmission of light at the appropriate wavelength. The solid-support is preferably suspended in a suitable liquid and the suspension is preferably agitated by passing a slow stream of gas bubbles through the capsule. By controlling the intensity of light delivered to the capsule and the duration of irradiation, the extent of cleavage of the support-bound material may be closely controlled. Where this technique is to be used routinely for all the capsules in the apparatus, the light-source for irradiation may be incorporated into the apparatus as an integral feature, after the manner of Figure 33, and the material cleaved from the support may be analysed using a flow-cell in the drain tubing, as previously described. This obviates the requirement to remove capsules from the apparatus for monitoring.

After removal of a capsule from the apparatus for analysis of its contents, the capsule may be returned to the apparatus in order to perform further synthetic operations. These may include repeating synthetic operations which have been performed previously but which have not yielded satisfactory results, as demonstrated by the analytical results obtained from the monitoring means. Alternatively, where the analytical results indicate that the contents of the capsule have been irreversibly degraded or converted to an unwanted product, the capsule may be discarded and a new capsule fitted to the apparatus in order to repeat the previous synthetic operations under different conditions.

By repeated application of the processes of synthesis and analysis, the apparatus may determine the optimum conditions for performing a particular synthetic

operation or series of operations.

Where required, the apparatus may be used in combination with a second apparatus to further increase the number of possible synthetic operations. Thus, capsules may be removed from a first apparatus in order to carry out further synthetic operations in a second apparatus before being replaced in the first apparatus. In this way it is possible to generate very large numbers of different chemical compounds in an automated system.

As will be apparent to those skilled in the art of synthesis automation, further features may be added to the apparatus to streamline operation or to detect malfunctions. For example, the correct operation of the actuators may be confirmed by adding sensors to the apparatus to detect proper positioning of the capsules after each step in the repositioning process. These sensors may, for example, comprise optical detectors or mechanically-operated microswitches attached to the guide rails of the apparatus or to appropriate points on the base plate. Alternatively, where the capsules contain embedded magnets as described in the foregoing embodiments, reed switches or Hall-effect switches may be used to verify the position of the capsules. Such sensors may be connected by a suitable interface to the control computer so that operations may be halted in the event of a malfunction.

As will also be apparent to the artisan, a variety of means may be employed to remove capsules from and replace capsules in the apparatus. As described previously, this means may comprise a robotic arm, many types of which are readily available from commercial sources. Alternatively, further actuators may be provided to lift the capsules or transfer capsules to a conveyor system. Where the capsules contain embedded magnets as described in the foregoing embodiments, magnetic means for lifting or conveying capsules may be employed in place of a robotic arm. It will also be apparent to the artisan that, where capsules contain magnetically operable valves, as described in the foregoing embodiments, a variety of means may be employed for applying an external magnetic field to the capsules in order to open the valves to allow passage of fluids through the capsules, including permanent magnets or electromagnets which may be fixed to the pressure-plate or the base-plate of the apparatus or may be movable.

As will also be apparent to the artisan, where capsules are heated or cooled during

the performance of chemical synthetic processes, a variety of heating or cooling means may be employed.

Although the apparatus described employs capsules of cuboidal form and movement means which moves the capsules through a rectangular path, it will be understood that other forms of capsule and movement means may equally be employed, for example cylindrical capsules or capsules of hexagonal prismatic form moving through a circular path would provide an equally effective apparatus.

It will also be understood that, although the apparatus as described contains positions for eight capsules, the number of capsules in the device may be increased or reduced according to the requirements of the specific application, with appropriate modification to the movement means.

As will be apparent to those skilled in the art of synthesis automation, the construction and layout of the valve systems and fluid-delivery systems supplying the fluid-delivery ports of the apparatus may take many different forms, depending on the nature of the fluids to be delivered and the volume and flow-rates of such liquids required in order to perform the desired synthetic operations.

Such systems may include pressurised solvent and reagent reservoirs, pumps, including syringe pumps, peristaltic pumps and piston pumps, vacuum systems to draw liquids through the capsules, or other fluid-movement means. Valve systems for selecting the fluids to be delivered may include solenoid valves, which may be two-way or three-way valves or may be mounted in a valve block, slider valves, rotary valves which may be motorised or pneumatically actuated and other types of valves. All such components are preferably controlled by a single controller, for example a computer with appropriate control program software and an appropriate interface board.

A particularly useful system which allows a large number of different fluids to be selected makes use of a valve block containing multiple solenoid valves which allows individual streams of fluid to be selected from pressurised reservoirs and linked to a common conduit for delivery to the reaction capsules. Such valve blocks have been described by Graffunder in U.S. Patent No. 4,168,724 and by Wittmann-Liebold in U.S. Patent No. 4,008,736 and a valve block of this type with ten solenoid valves is commercially available from Perkin Elmer/Applied Biosystems Inc. (Part No. 602251). Using valve blocks of this type in conjunction

with pressurised fluid reservoirs, a fluid-delivery system may be constructed in which each fluid delivery port may be supplied with any of ten different fluids or a combination thereof. In the apparatus previously described, which has eight fluid-delivery ports, the system therefore allows any of eighty different fluids to be supplied to the reaction capsules. Figure 34 shows diagrammatically the plumbing of this apparatus, which provides a highly flexible system for performing many different types of chemical synthesis. Specific examples of applications of the apparatus will now be described, by way of illustration.

Example 1 : Oligonucleotide Synthesis

The apparatus may be applied to the synthesis of oligonucleotides, which are very important in many fields of biological and biochemical research. A particular requirement in oligonucleotide synthesis is the ability to synthesise large numbers of different oligonucleotides automatically in a single system without operator attention.

The chemical intermediates employed in oligonucleotide synthesis are well known to those skilled in the art, and are readily available from commercial sources. They include cyanoethyl phosphoramidites of the four common deoxyribonucleosides deoxyAdenosine (dA), deoxyGuanosine (dG), deoxyCytidine (dC) and Thymidine (T) as well as of the four common ribonucleosides Adenosine (rA), Guanosine (rG), Cytidine (rC) and Uridine (U). In addition, cyanoethyl phosphoramidites of many modified nucleosides are available. Some of these modified nucleosides are minor constituents of natural DNA or RNA, while others may be used to attach a label to an oligonucleotide molecule, for example a Biotin substituent. Yet other nucleosides contain fluorescent dye labels which are incorporated in oligonucleotides for use in automated DNA sequencers which determine the genetic sequence of natural genes.

Conventional oligonucleotide synthesisers have a limited number of reagent reservoirs capable of containing these modified nucleosides. Typically, between one and four reservoirs may be provided for such nucleosides in addition to the common nucleosides. This restricts the range of oligonucleotides which may be produced on the instrument without halting the instrument in order to change the reagents. This limitation does not apply to the apparatus of the invention, which is

capable of accommodating a large number of different reagents.

The process of preparing oligonucleotides commonly employs a cyclic procedure in which each cycle includes the operations of : (i) Deblocking, which removes the 5'-terminal protecting group from the solid-support-bound precursor, (ii) Coupling, in which the next nucleoside is attached to the solid-support-bound precursor, (iii) Capping, in which the unreacted sites on the solid-support are inactivated, and (iv) Oxidation, in which the phosphite-triester linkage generated during coupling is converted to a phosphotriester linkage. This cycle is repeated until the desired oligonucleotide sequence is complete, whereupon the oligonucleotide is cleaved from the solid-support and remaining protecting groups are removed.

The apparatus of the invention is capable of performing all the operations required to complete each cycle of an oligonucleotide synthesis. This may be achieved by connecting reagent reservoirs to the valve system supplying each of the eight fluid-delivery ports as follows:

Fluid-delivery Port 1:

valve port 1: dA phosphoramidite solution (0.1M in acetonitrile)
valve port 2: Activator solution (0.5M tetrazole in acetonitrile)
valve port 3: Wash solvent (acetonitrile)
valve port 4: Carrier Gas (Argon)
(remaining valve ports unused)

Fluid-delivery Port 2:

valve port 1: dG phosphoramidite solution (0.1M in acetonitrile)
valve port 2: Activator solution (0.5M tetrazole in acetonitrile)
valve port 3: Wash solvent (acetonitrile)
valve port 4: Carrier Gas (Argon)
(remaining valve ports unused)

Fluid-delivery Port 3:

valve port 1: dC phosphoramidite solution (0.1M in acetonitrile)
valve port 2: Activator solution (0.5M tetrazole in acetonitrile)
valve port 3: Wash solvent (acetonitrile)

valve port 4: Carrier Gas (Argon)

(remaining valve ports unused)

Fluid-delivery Port 4:

valve port 1: T phosphoramidite solution (0.1M in acetonitrile)

valve port 2: Activator solution (0.5M tetrazole in acetonitrile)

valve port 3: Wash solvent (acetonitrile)

valve port 4: Carrier Gas (Argon)

(remaining valve ports unused)

Fluid-delivery Port 5:

valve port 1: FAM phosphoramidite solution

valve port 2: HEX phosphoramidite solution

valve port 3: TET phosphoramidite solution

valve port 4: Fluorescein phosphoramidite solution

valve port 5: Rhodamine phosphoramidite solution

valve port 6: Texas Red phosphoramidite solution

valve port 7: TAMRA phosphoramidite solution

valve port 8: Activator solution (0.5M tetrazole in acetonitrile)

valve port 9: Wash solvent (acetonitrile)

valve port 10: Carrier Gas (Argon)

Fluid-delivery Port 6:

valve port 1: Cap A solution

valve port 2: Cap B solution

valve port 3: Wash solvent (tetrahydrofuran)

valve port 4: Carrier Gas (Argon)

(remaining valve ports unused)

Fluid-delivery Port 7:

valve port 1: Oxidising solution

valve port 2: Wash solvent (tetrahydrofuran)

valve port 3: Carrier Gas (Argon)

(remaining valve ports unused)

Fluid-delivery Port 8:

valve port 1: Deblock solution

valve port 2: Wash solvent (dichloromethane)

valve port 3: Carrier Gas (Argon)

(remaining valve ports unused)

In the above description, the terms FAM, HEX, TET, Fluorescein, Rhodamine, Texas Red and TAMRA refer to fluorescent dyes which may be attached to oligonucleotides for use in DNA sequencers, Cap A solution is a solution of Acetic Anhydride in tetrahydrofuran/2,6-lutidine, Cap B solution is a solution of N-methyl-imidazole in tetrahydrofuran, Oxidising solution is a solution of Iodine in tetrahydrofuran/pyridine/water, and Deblocking solution is a solution of trichloroacetic acid in dichloromethane.

In addition to the above reagents, a supply of reaction capsules pre-loaded with solid-support is required. The solid-support carries a covalently-linked nucleoside which serves as the 3'-terminus of the oligonucleotide to be synthesised. There are thus 4 different types of support, corresponding to the 4 natural deoxyribonucleosides dA, dG, dC and T. Other types of support may be used, for example for RNA synthesis or where a 3'-linked modified nucleoside or label is required. In addition, supports may have various levels of loading of nucleoside on the solid-support, and the internal volume of the reaction capsule, and thus the volume of solid support, may be increased or decreased depending on the quantity of oligonucleotide to be synthesized. Since each capsule carries an individual bar-code label or RF-tag by means of which it may be identified, a comprehensive range of reaction capsules of many different types may be prepared in advance and held in a storage system until required. The appropriate type of capsule for any particular synthesis may thus be selected and loaded into the apparatus automatically, for example using a robotic arm, by sending appropriate signals or commands from the control computer.

In order to perform oligonucleotide synthesis using the apparatus, the sequence data for the oligonucleotides required is first loaded into the control computer. The computer then issues instructions for retrieving appropriate reaction capsules from the storage system and loading them into the apparatus, for example using a robotic arm. When one or more capsules of the apparatus is not in use, dummy capsules must be fitted to the unused positions in order that the movement mechanism will still function correctly. Since the apparatus is intended for continuous use, all of the capsule positions will normally be occupied, and a new

capsule may only be fitted after one of the existing capsules is removed from the apparatus on completion of synthetic operations in that capsule. However, when the apparatus is set in operation for the first time, all of the available capsule positions will necessarily be occupied by dummy capsules. In either case, each new synthesis is initiated by removing a capsule from the apparatus, selecting an appropriate capsule for the new synthesis to be commenced, and fitting this capsule to the vacant space in the apparatus. All of these operations may be performed automatically under instructions from the control computer. The control computer then issues commands to the movement mechanism to move all the capsules into alignment with the fluid-delivery and fluid-collection ports, following which the compression means is activated in order to create a seal between each capsule and its complementary ports.

Once the capsules have been sealed, commands can be issued by the control computer to the valves in order to deliver chemicals to each capsule as appropriate. For example, a capsule connected to fluid-delivery port 1, as listed above, may be supplied with a mixture of dA phosphoramidite solution and activator solution in order to couple a dA monomer unit to the oligonucleotide being synthesised in that capsule. Alternatively, if another monomer is required at that point in the synthesis of the oligonucleotide being synthesised in that capsule, no solutions will be delivered to the capsule while it is in that position. Meanwhile, the valves connected to the other capsules are controlled in similar fashion. For example, the capsule connected to fluid-delivery port 8, as listed above, may require a deblocking operation at that point of the synthesis. Thus, the computer will open the appropriate valve connected to that port for an appropriate period, following which wash solvent may be delivered to the capsule by closing that valve and opening another. When all the chemical operations required in all capsules have been completed, residual fluids may be flushed out of the capsules by delivering carrier gas to the capsules. Following this, the compression means may be deactivated, lifting the pressure-plate and releasing the capsules, allowing them to be moved into new positions. The control computer then issues instructions appropriate to the next stage of the syntheses. This cycle of operations is continued until all the syntheses required in a particular capsule have been completed. This capsule may then be removed and replaced with a new capsule, if

further syntheses are still to be commenced, or with a dummy capsule if no further syntheses remain to be commenced.

Capsules in which syntheses have been completed may be transferred to a second apparatus in which appropriate reagents may be delivered in order to cleave the completed oligonucleotide from the solid-support and remove remaining protecting groups. Since these operations usually require more time for completion than the actual synthesis of the oligonucleotide, transferring the capsules to a second apparatus allows the first apparatus to operate more efficiently.

The apparatus has several advantages over conventional instruments for oligonucleotide synthesis. Firstly, it is able to synthesise large numbers of oligonucleotides by continuous operation, while retaining the ability to control each synthesis individually and tailor reaction conditions for each synthesis. In this respect, it is superior, for example, to instruments using multi-well plates, in which all syntheses must be carried out under similar conditions. Because the apparatus of the invention carries out synthetic operations on each capsule individually and asynchronously from other capsules, it is much more flexible and versatile than multiple parallel synthesisers, allowing syntheses of widely different scales and using a large number of different monomer units to be carried out simultaneously in the same apparatus.

Secondly, because each fluid-delivery port in the apparatus may be dedicated to a particular reagent, the apparatus is economical in operation, since the tubing between valves and capsules may be very short and does not require prolonged washing to avoid cross-contamination with other reagents. This also provides the opportunity for recycling reagents and solvents, since the waste from each capsule position is collected separately rather than being mixed together. Thus, nucleoside monomers may be recovered and recycled without risk of cross-contamination, and capping, oxidising and deblock solutions may be re-used or recycled. Since the cost of waste solvent and reagent disposal constitutes a major element in the operating costs of an oligonucleotide synthesiser, this ability represents an important cost saving, in addition to the environmental benefits obtained.

Thirdly, as the capsules may be relatively large, the apparatus provides the capacity for producing large quantities of oligonucleotides by repeated synthesis

of the same oligonucleotide in multiple capsules. A particular advantage of this approach over conventional large-scale synthesisers is that synthesis progress in each capsule may be monitored and the products from each capsule subjected to individual analysis before combining the products from satisfactory syntheses. The risk of synthesis failure is thereby reduced, and compliance with regulatory requirements, for example prior to clinical trials, is facilitated. The design of the capsules and their suitability for robotic handling allows automation of the analytical procedures involved, and the capacity for recovery and recycling of monomers and reagents reduces the cost of a large-scale synthesis.

Example 2 : Peptide Synthesis

The apparatus may also be applied to the synthesis of peptides, which are widely used in biological and biochemical research and also have important industrial applications, for example in vaccine production. A particular difficulty in peptide synthesis is the ability to accommodate all of the naturally-occurring amino-acids and to tailor synthesis conditions according to the amino-acid employed in a particular step of a synthesis. In both these respects, the apparatus of the invention offers advantages over conventional peptide synthesizers.

The chemical intermediates employed in peptide synthesis are well known to those skilled in the art, and are readily available from commercial sources. They include N-Fluorenylmethoxycarbonyl (FMOC) and N-tert-Butoxy-carbonyl (t-BOC) amino-acids and activated derivatives thereof, for example 4-nitrophenyl and pentafluorophenyl esters. In addition, many amino-acids exist in both D- and L- forms, and modified amino-acids such as halo-substituted phenylalanines, thienyl-alanine, naphthyl-alanine and pyridyl-alanine may also be used in peptide synthesis. Conventional synthesizers do not have sufficient reagent reservoirs to accommodate all of the possible amino-acid derivatives which may be required in a synthesis. The apparatus of the invention does not have this shortcoming and, further, allows different reaction conditions to be applied to each coupling step in a synthesis. Furthermore, the course of the coupling reaction may be monitored in order to maximise the yield of product. The apparatus also allows double coupling reactions to be performed in some capsules without interrupting the syntheses taking place in other capsules.

In the apparatus as described previously, the reagent reservoirs associated with one capsule position and valve block may be allocated to deblocking solution, wash solvent and carrier gas. For example, where FMOC amino acids are to be used, the deblocking solution may be a solution of piperidine in dimethylformamide (DMF), the wash solvent may be DMF, and the carrier gas may be Argon. The reagent reservoirs associated with other valve blocks are allocated to amino-acid derivatives and activator solutions.Where required, two positions of each valve block may be allocated to activating solutions, for example a solution of di-isopropyl carbodiimide in DMF, and a solution of 1-hydroxy-benzotriazole in DMF, and one position each to wash solvent (DMF) and carrier gas. This leaves six positions at each valve block for amino-acid derivatives, allowing forty-two different amino-acid derivatives to be employed in a synthesis.

In order to perform peptide synthesis using the apparatus, the sequence data for the peptides required is first loaded into the control computer. The computer then issues instructions for retrieving appropriate reaction capsules from the storage system and loading them into the apparatus, for example using a robotic arm. As described in the preceding example, these contain solid-support to which is covalently linked the C-terminal amino acid of the required peptide sequence.

The control computer then issues commands to the movement mechanism to move all the capsules into alignment with the fluid-delivery and fluid-collection ports, following which the compression means is activated in order to create a seal between each capsule and its complementary ports.

Once the capsules have been sealed, commands can be issued by the control computer to the valves in order to deliver chemicals to each capsule as appropriate. In the case of a newly-initiated synthesis, this will usually require deblocking of the amino-acid by delivering deblocking solution to the capsule. Following this, wash solvent is delivered to the capsule. Finally, carrier gas is delivered to the capsule to flush out remaining liquids. After releasing the pressure-plate, capsules may be moved to a new position before reactivating the compression means. If the amino-acid required in each capsule is available from the valve block connected to the corresponding fluid-delivery port, it may then be delivered to the capsule, together with activating solutions where required. The course of the coupling reaction may be monitored, for example by recirculating

the solution through the capsule and monitoring the absorption or fluorescence of the solution in a flow-cell, in order to measure the decrease in concentration of the activated species as the coupling proceeds. When the coupling reactions are complete in all capsules, wash solvents and carrier gas may be delivered to the capsules as previously described, before releasing the pressure-plate and proceeding to the next step of the synthesis.

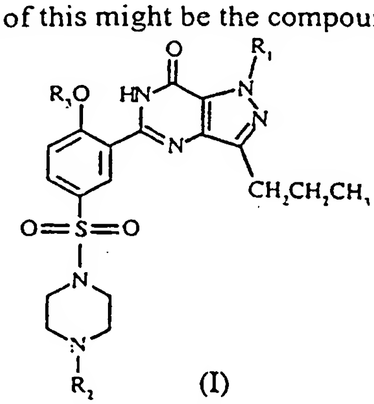
As an alternative to monitoring the course of the coupling reaction by recirculation through a flow-cell, where the capsules are equipped with internal valves and windows as described in the preceding embodiments, the course of the reaction may be followed by removing each capsule from the apparatus to a second apparatus adapted to monitor coupling reactions. Other capsules may then be fitted to the apparatus to commence new syntheses while the coupling reactions in the original capsules are still in progress. This enhances throughput of the system.

If it becomes apparent from the monitoring of a coupling reaction that it is unlikely to reach completion, the control computer may automatically initiate a second attempt at a coupling reaction, or "double coupling".

After the completion of all synthetic operations required in a capsule, the capsule may be removed, for example by a robotic arm, and transferred to a second apparatus in which the product peptide may be cleaved from the solid-support and remaining protecting groups removed.

Example 3 : General Combinatorial Synthesis

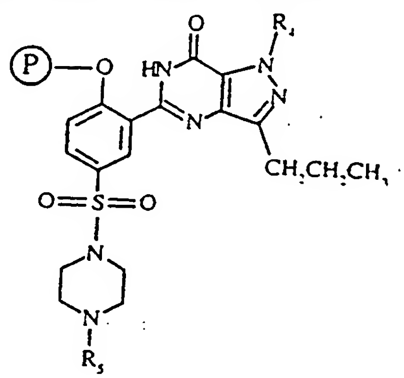
The apparatus may be employed in the synthesis of large numbers of analogues of a particular chemical structure. For instance, it might be desired to synthesise analogues of a compound containing acyl substituents at two different positions in the molecule. An example of this might be the compound (I):



(I)

Compound (I), where R_1 and R_2 are methyl and R_3 is ethyl, has known activity as a selective inhibitor of cyclic guanosine 3',5'-monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) and has attracted considerable interest as an oral therapeutic for erectile dysfunction (VIAGRA®). However, the original intention of the researchers who discovered this compound was to develop therapeutic anti-anginal agents. It might be speculated that introduction of a variety of acyl groups in place of R_1, R_2 and R_3 would alter the spectrum of activity and provide further compounds of therapeutic utility. The apparatus of the invention may be applied to the synthesis of such analogous series, as follows:

Taking the example of the above compound, the first step required is the synthesis of a solid-support bound molecule containing the backbone of the molecule. Such a compound may be represented by the structure (II):



(II)

in which (P) represents a polymer support to which the molecule is covalently attached by a cleavable linkage, and R_4 and R_5 are protecting groups which are independently removable without affecting the linkage to the polymer support.

In a first phase of the synthesis, capsules containing this polymer supported molecule are assembled and individually loaded into the apparatus of the invention, in which each of the valve blocks has one position allocated to a wash solvent and another valve position allocated to carrier gas. One other valve block additionally has one position allocated to a deprotection reagent specific for removal of the protecting group R_4 and another valve block has one position

allocated to a deprotection reagent specific for removal of the protecting group R_5. The remaining 62 positions of all valve blocks may be allocated to acylating reagents, each of a different structure.

Capsules are introduced to the apparatus as in the preceding examples, and, as each capsule reaches the appropriate position, it is treated with the deprotection reagent specific for removal of the protecting group R_4. After washing and flushing out residual liquids with carrier gas, the pressure-plate is released and the capsules are moved to new positions before reactivating the pressure-plate, as described in the foregoing examples. As each capsule reaches an appropriate position, one of the acylating reagents is introduced and the extent of acylation is monitored until the reaction has reached completion. After another washing and flushing cycle, the capsules are moved to new positions, but no further solutions are delivered to capsules which have been treated with the first acylating reagent until they reach the position at which they can be treated with the deprotection reagent specific for removal of the second protecting group R_5. At this point, each capsule is treated with this deprotection agent, again washed and flushed with carrier gas, and the capsules again moved after releasing the pressure-plate. When each capsule reaches the position corresponding to the second acylation reagent required for the synthesis of the analogue in that capsule, the reagent is delivered to that capsule, and the extent of acylation is again monitored until the reaction has reached completion. After a further washing and flushing cycle, synthetic operations in that capsule have been completed, so that, when it next reaches the appropriate position in the apparatus, it may be removed and replaced with another capsule in which a different analogue is to be synthesised.

By repetition of this cycle, all of the possible compounds containing combinations of the substituents R_2 and R_3 may be synthesised individually. In the example given, a total of 3844 different analogues is possible. Each capsule circulates in the apparatus at least once and possibly up to four times before the appropriate combination of reagents is obtained.

Where monitoring of the extent of reaction in a capsule indicates that the reaction has not proceeded to completion, the capsule may be replaced with another capsule and the reaction repeated under a different set of conditions. This cycle may be reiterated until a satisfactory result is obtained for all capsules.

Alternatively, the products of reactions which have not proceeded satisfactorily may be excluded from subsequent assays in order to avoid generating spurious assay results.

After completion of all syntheses, the analogues may be cleaved from the support and collected. During this process, a further substituent may be introduced at the cleavage site, where this is compatible with the cleavage reagent. Multiple runs of the apparatus may be performed, with different substituents being introduced at the cleavage step, thus further increasing the number of possible combinations.

Where the capsules are equipped with windows and the linker to the solid support is cleaved by photochemical means, as described previously, a further enhancement is possible, in that aliquots of each of the compounds synthesised may be cleaved from the solid support and automatically transferred to another apparatus for an assay procedure, for example using a robotic system. The material remaining bound to the solid support may be stored in the capsule and may be transferred to an automated storage system from which it may subsequently be retrieved using the bar-code or RF-tag for identification when next required. This facilitates the handling and storage of large numbers of compounds in automated assay procedures and also allows compounds of widely-differing structures to be brought together in a common assay, enabling structure-activity data to be obtained over a large range of different chemical compounds.

The example given here is illustrative, and to some extent atypical in that both of the substituents introduced to the molecule are of the same chemical type, namely acyl groups. In general, where combinations of two substituents are required, it is likely that the groups will be of a different chemical nature. In this case, the available reagent reservoirs of the apparatus must be divided between two groups of reagents, thus reducing the number of possible combinations. In the case of the apparatus as described, containing eight valve blocks each with ten valves, the maximum number of combinations of two substituents is 6,400 where the substituents are of the same type. This is reduced to 1,600 where the substituents are of different types.

The number of possible combinations of substituents may be dramatically increased, however, by using two or more instruments, each of which may accommodate either the same or different types of substituent, and transferring

capsules between the instruments, for example by use of a robotic arm. This process is illustrated diagrammatically in Figure 35, which represents the situation where two instruments are involved. The total number of possible combinations in this case is 25,600 where the substituents are of the same type and 6,400 where the substituents are of different types.

An extension of this process is illustrated diagrammatically in Figure 36, which represents the situation where three instruments are involved, each of which may introduce a different type of substituent. Transfer of capsules between all three instruments allows a total of 512,000 possible combinations of three substituents.

Where a single apparatus is employed, the number of possible combinations may also be increased by adding extra valves to the apparatus or by increasing the number of fluid-delivery and fluid-collection ports in the apparatus.

As will be apparent to those skilled in the art of combinatorial chemical synthesis, the handling of such large numbers of reaction capsules requires a high level of automation. The construction of the capsules lends itself to such automation, since the capsules are readily handled, for example, by robotic systems. The capsules may also be constructed from materials which are suitable for injection-moulding, for example polypropylene. In this case, the sealing O-rings may be integral with the capsules, and the capsules may be mass-produced.

The internal volume of the capsules may be increased or decreased according to requirements. Thus, in very small scale syntheses, the volume of the reaction chamber may be as small as 10 microlitres or less, whereas larger capsules having a volume of 100 millilitres or more may be appropriate for large-scale syntheses.

A particular advantage of the design of the apparatus is the ability to accommodate capsules with different internal volumes but identical external dimensions in a single apparatus, thus providing flexibility of synthesis scales.

The method of loading the solid-support material in the capsules may also be automated, for example by dispensing the solid-support into the capsules as a slurry in a suitable liquid. Where the support material has sufficiently low density, an automatic pipettor may be used to dispense the material in dry form. Electrostatic attraction may also be used to handle and dispense dry solid-support material into the capsules. Alternatively, where a fibrous material may be used as the solid-support, this may simply be packed into the reaction chamber. In this

case, the retaining filters may not be required.

The attachment of the substrate molecule to the solid support may be performed on a large scale before the resulting solid-support is distributed into the reaction capsules. This will usually be the case where oligonucleotides or peptides are to be prepared. However, where combinatorial synthesis of large numbers of structural analogues is required, the attachment process may also be performed in the apparatus, thus presenting a further opportunity to diversify the range of possible compounds. In this approach, the capsules are first loaded with a solid-support which includes an attachment site, and a variety of different chemical species which are capable of reaction with the attachment site are loaded, in solution, into the reagent reservoirs of the apparatus. The capsules are then passed through the apparatus as previously described, and the appropriate solution is delivered to each capsule. This process provides a set of capsules containing solid-supports with a diverse range of attached structures. These capsules may then be transferred to a second apparatus which introduces a second substituent, thus increasing the structural diversity. This cycle may be repeated in a third instrument which introduces a third substituent, thus increasing the structural diversity still further.

Conclusion

The foregoing description illustrates embodiments of the invention. However, the invention is not restricted to the specific embodiments described, and many variations of the invention will be apparent to those skilled in the art of combinatorial chemical synthesis. Accordingly, the scope of the invention should not be determined by reference to the foregoing description, but solely by reference to the appended claims along with their full scope of equivalents.

CLAIMS

1. A method for performing in synthesis equipment one or a multiplicity of combinatorial chemical syntheses on at least one capsule, each capsule containing a solid-support, the method comprising placing a capsule at a first station in the synthesis equipment for synthesis to be effected at that station, activating compression means to create a fluid-tight seal between a delivery port of fluid-dispensing means and an inlet conduit of the capsule at the station, delivering fluid to the capsule for a first synthesis operation in the capsule, and deactivating the compression means to allow the capsule to be moved to a second station of the synthesis equipment.

2. A method according to Claim 1 characterised by activating compression means to create a fluid-tight seal between a collection port and an outlet conduit of the capsule at the station.

3. A method according to Claims 1 or 2 characterised by moving the capsule successively to a plurality of further stations at which sealing activation/deactivation operations and fluid-delivering operations occur.

4. A method according to any preceding claim, characterised by moving the capsule to at least one station at which no sealing activation/deactivation operation and no fluid-delivery operation occur.

5. A method according to any preceding claim characterised by moving the capsule to a station at which the capsule is removed from the synthesis equipment.

6. A method according to Claim 5 characterised by introducing a capsule to a station of the synthesis equipment to replace a capsule which has been removed from the synthesis equipment.

7. A method according to Claim 6 characterised by introducing a capsule to the same station from which a capsule had previously been removed.

8. A method according to any preceding claim characterised by monitoring the progress of any chemical process taking place in a capsule at one or more stations.

9. A method according to Claim 8 characterised by making alterations, in dependence on the monitoring step, to the quantity, and/or rate of delivery and/or identity of fluids delivered to a capsule during synthesis operations.

10. A method according to Claims 8 or 9 characterised by varying, in dependence on the monitoring step, the temperature of a capsule at a station.

11. A method according to any of Claims 8 to 10 characterised by re-iterating one or more steps until no further reaction is detected.

12. A method according to any preceding claim characterised by simultaneously operating compression means at a plurality of stations.

13. A method according to any preceding claim characterised by moving a plurality of capsules between stations after one deactivation

operation of compression means and before the next activation operation of compression means.

14. A method according to any preceding claim characterised in that the synthesis products are oligonucleotides or polynucleotides.

15. A method according to any of Claims 1 to 13 characterised in that the synthesis products are oligopeptides or polypeptides.

16. A computer program product directly loadable into the internal memory of a digital computer, comprising software code portions for performing the method of any one or more of Claims 1 to 15 when said product is run on a computer.

17. A computer program product stored on a computer usable medium, comprising:

a) computer readable program means for causing a computer to place a capsule at a first station;

b) computer readable program means for causing the computer to activate compression means to create a fluid tight seal between a delivery port of fluid dispensing means and an inlet conduit of the capsule at the station;

c) computer readable program means for causing the computer to deliver fluid to the capsule for a first synthesis operation in the capsule;

d) computer readable program means for causing the computer to deactivate the compression means to allow the capsule to be moved to a second station of the synthesis equipment.

18. Electronic distribution of a software program according to Claim 16 or 17.

19. A reaction capsule for use in automated single or multiple combinatorial chemical synthesis, the capsule comprising a reaction zone to contain a solid-support, a inlet conduit for the passage of fluids through the reaction zone, means to form a fluid-tight seal between the inlet conduit and a fluid delivery port of a fluid-dispensing means, said fluid-tight seal means being activated solely by the application of pressure between said capsule and said port.

20. A capsule according to Claim 19 characterised by the capsule having means to form a fluid-tight seal between an outlet conduit of the capsule and a fluid-collection means.

21. A capsule according to Claims 19 or 20 characterised by the inlet seal means and/or the outlet seal means being deactivated in the absence of applied pressure between said capsule and said fluid-dispensing means or said fluid-collections means, as appropriate.

22. A capsule according to any of Claims 19 to 21 characterised by the sealing means being operable by means to activate a plurality of capsules simultaneously.

23. A capsule according to any of Claims 19 to 22 characterised by an inlet port and an outlet port being located on the same face of the capsule.

24. A capsule according any of Claims 19 to 23 characterised by the capsule having means to effect automatic monitoring of chemical processes taking place within the capsule.

25. A capsule according to Claim 24 wherein the means to effect automatic monitoring comprises transparent areas of side walls of the capsule.

26. A capsule according to Claim 25 wherein the means to effect automatic monitoring comprises a transparent area of a side wall and a reflection area of a side wall of the capsule.

27. A capsule according to any of Claims 19 to 26 wherein the capsule comprises machine-readable identification means.

28. Equipment for automated single or multiple combinatorial chemical synthesis, the apparatus characterised by a plurality of stations for capsules each containing a solid support for chemical synthesis, means to move the capsules between stations, means to activate and deactivate compression means of capsules at at least some of the stations, and means to deliver fluids to capsules at at least some of the locations.

29. Equipment according to Claim 28, characterised by means to collect fluid from capsules at at least some of the stations.

30. Apparatus according to Claims 28 or 29, characterised by means to activate and deactivate compression means of a plurality of capsules simultaneously.

31. Equipment according to any of Claims 28 to 30 characterised by means to automatically monitor chemical processes taking place in capsule(s) at one or more stations.

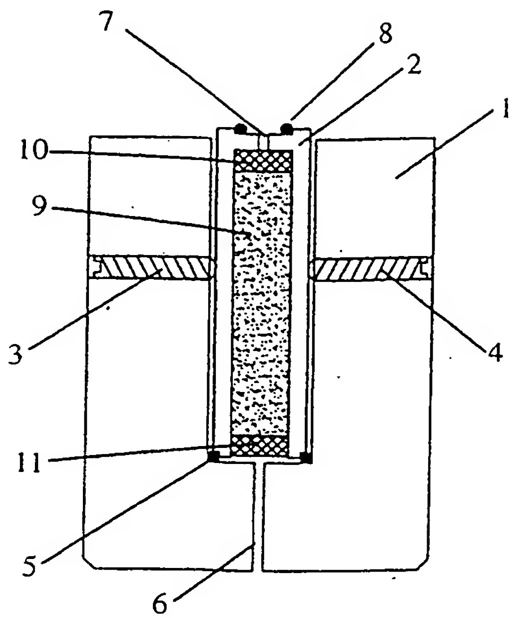


Figure 1

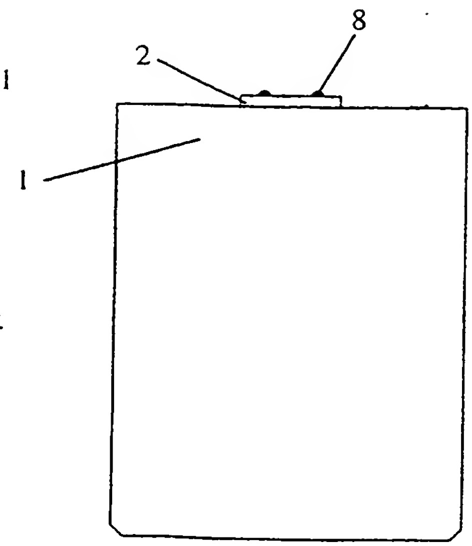
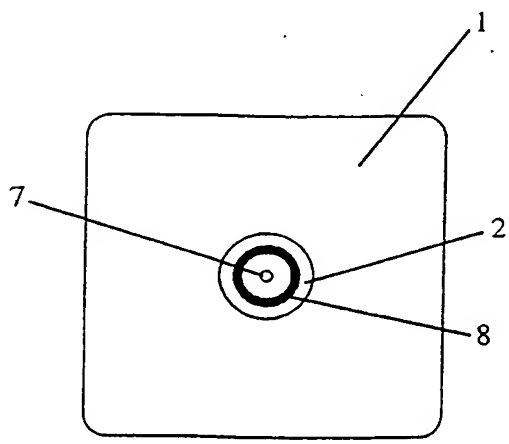


Figure 2



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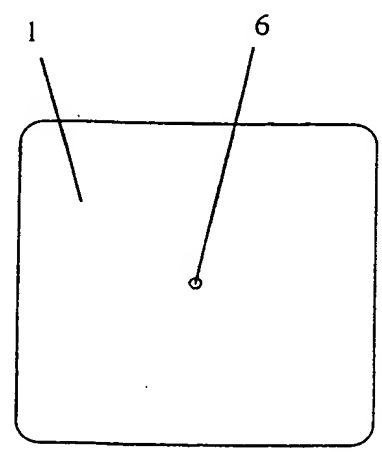

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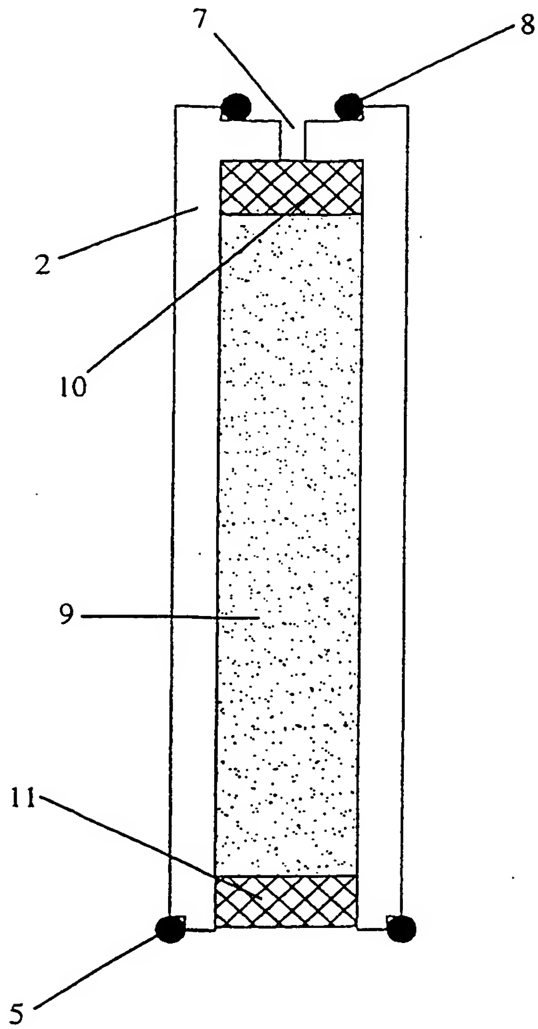

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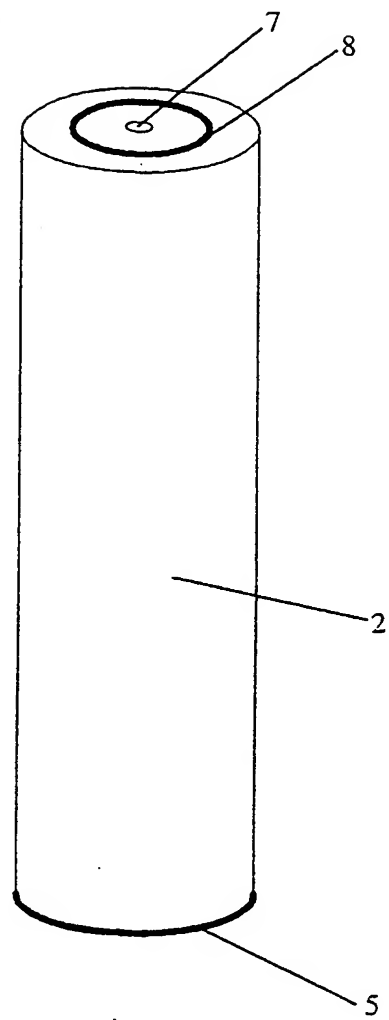

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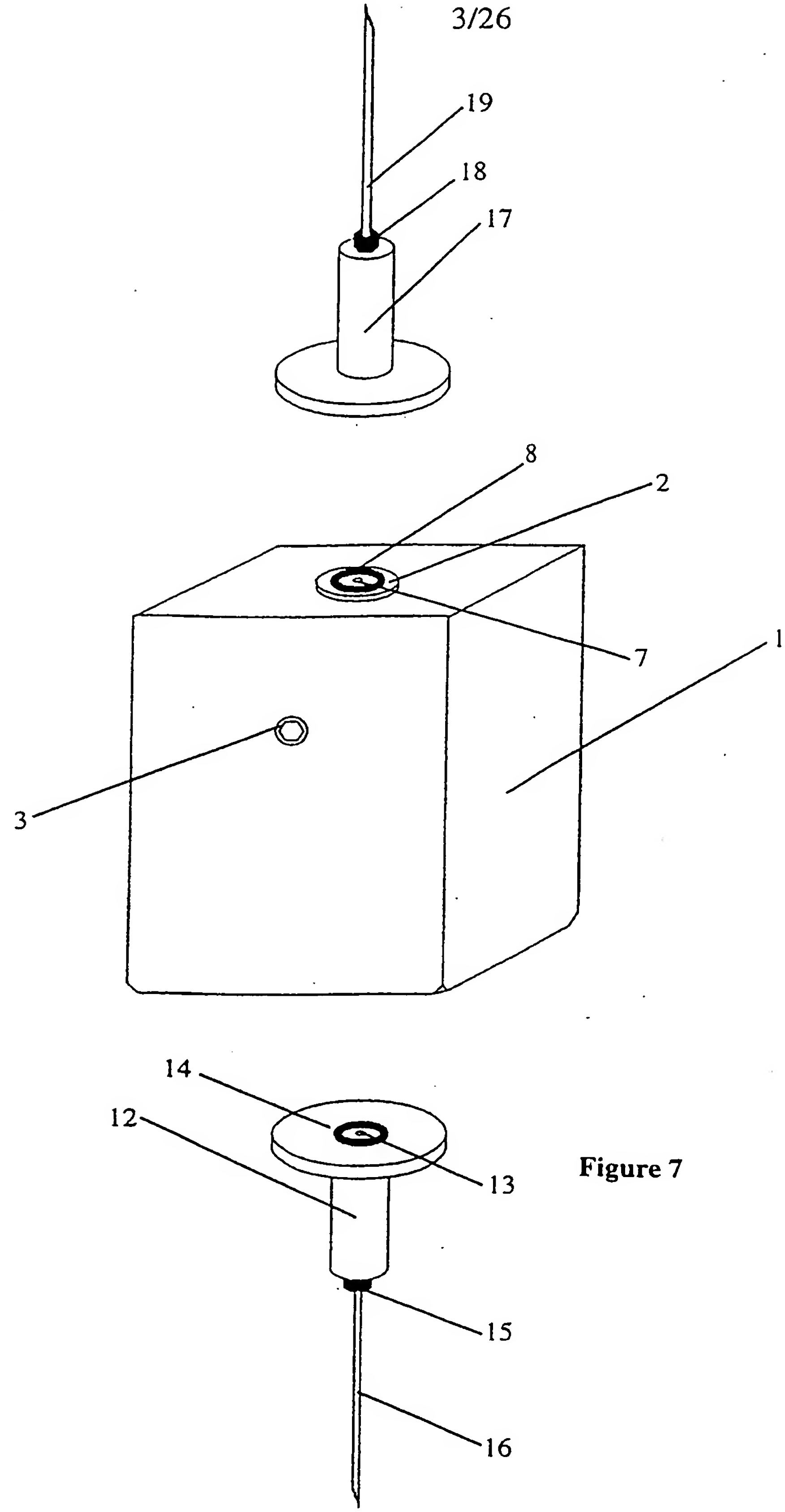

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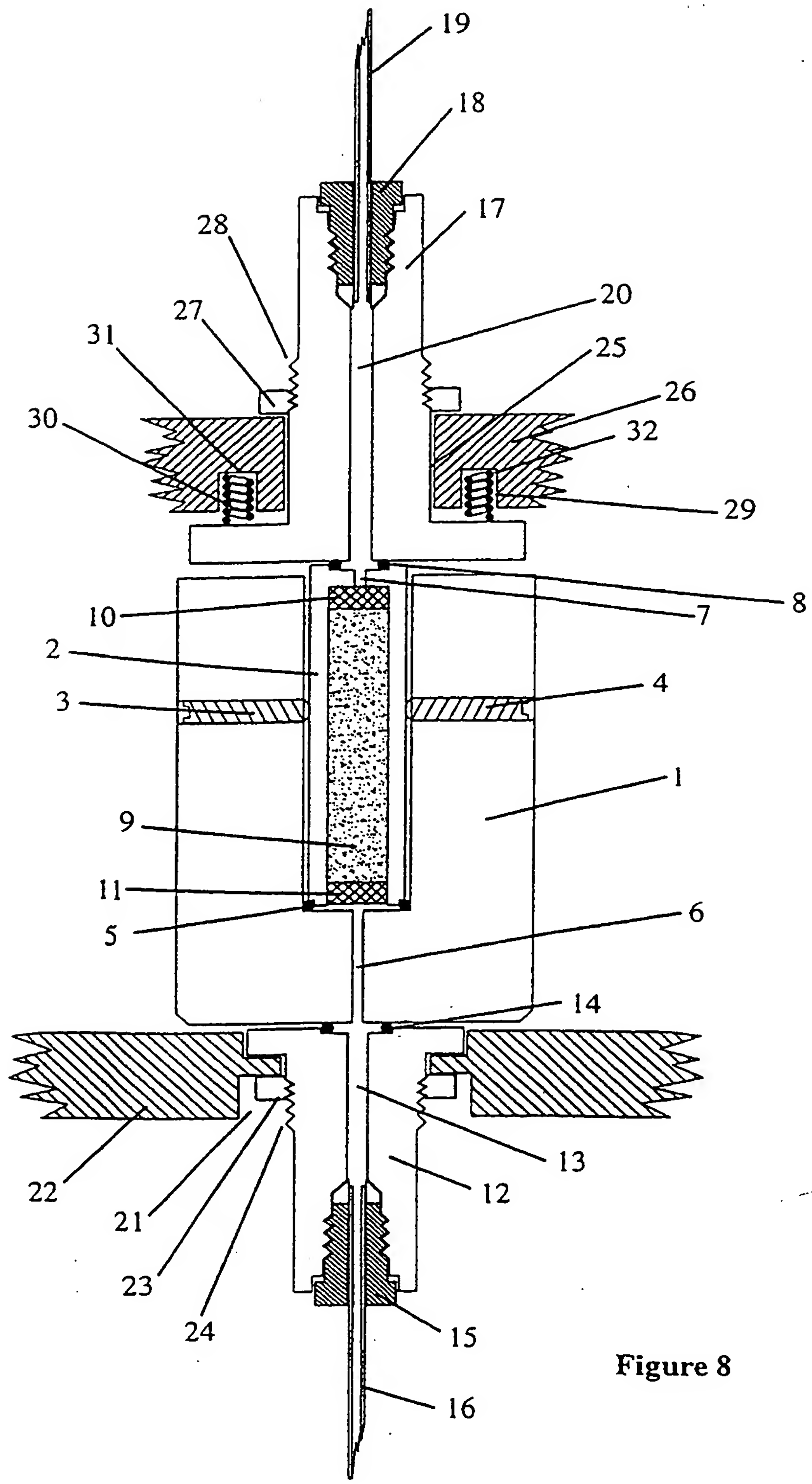
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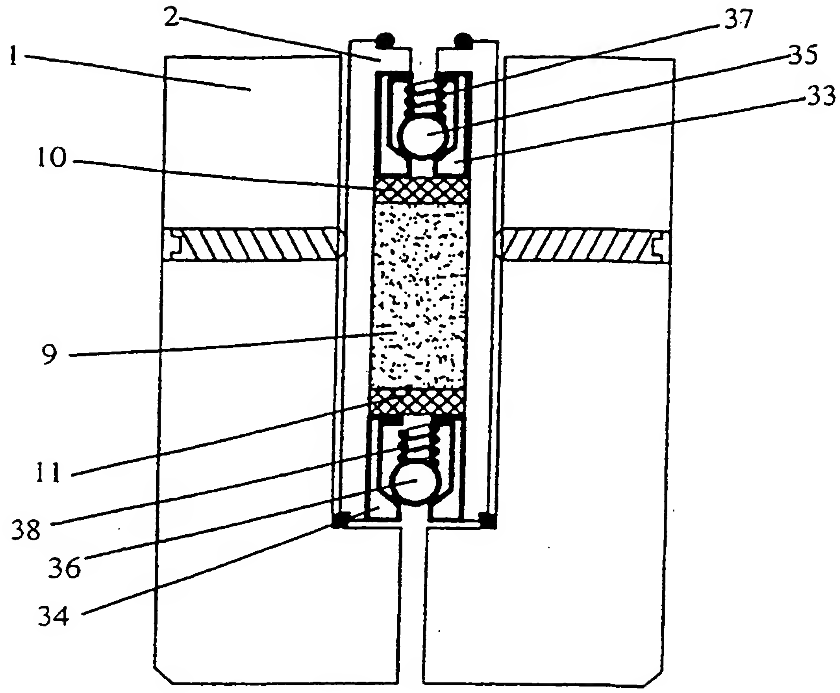
Figure 8



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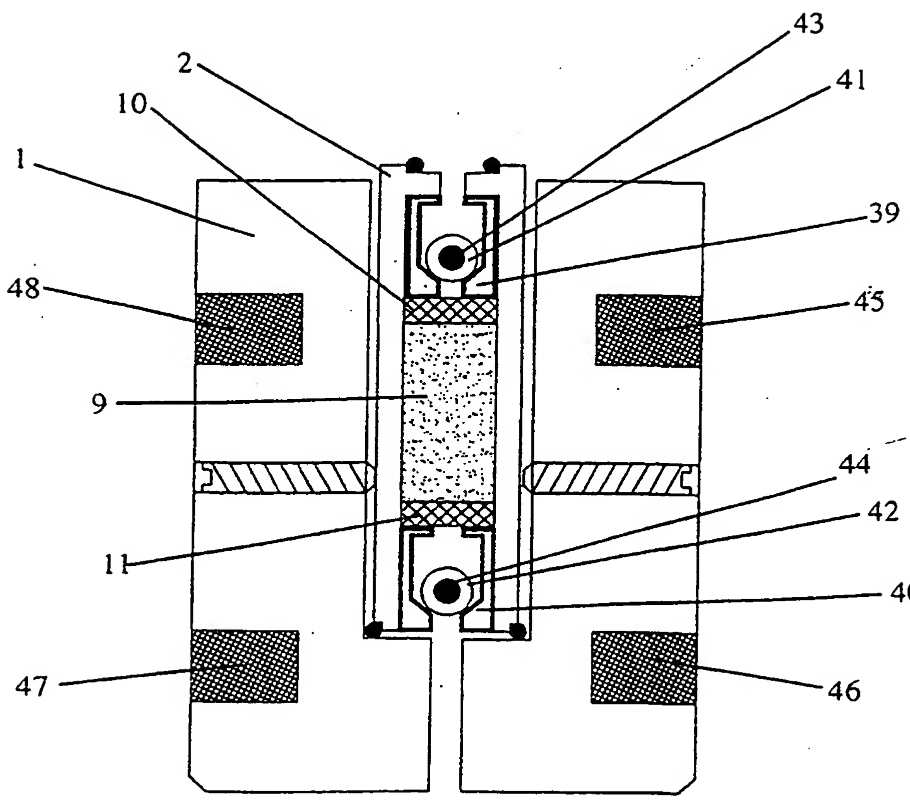

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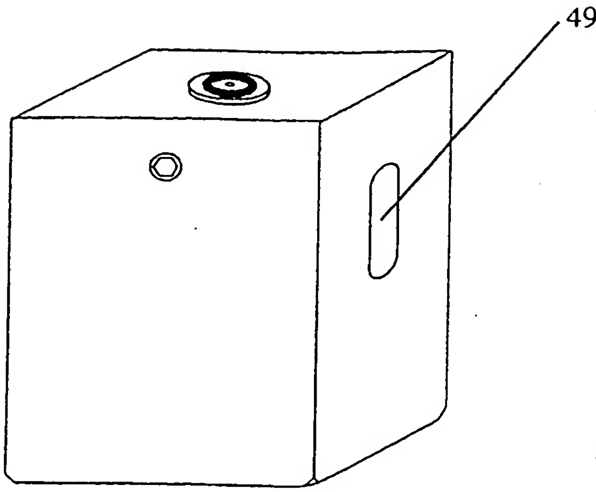

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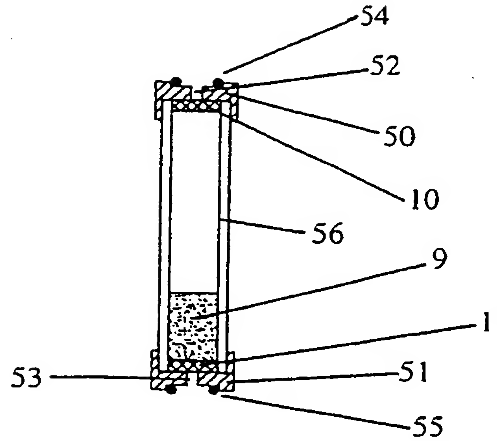

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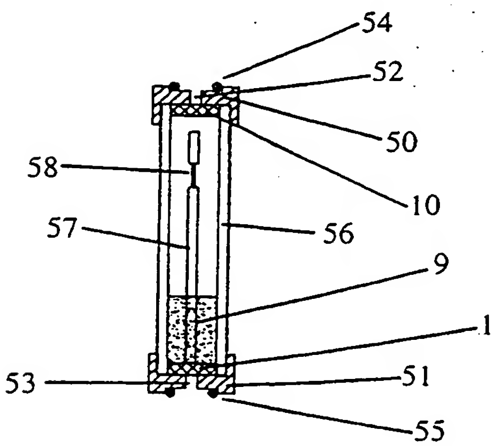

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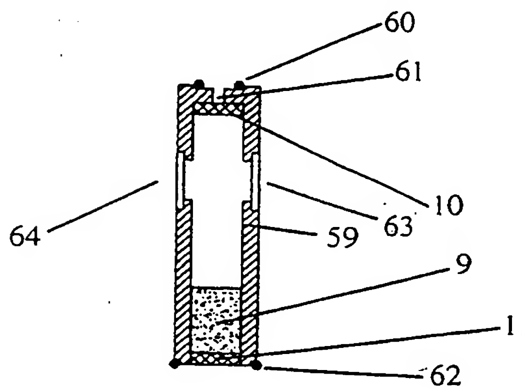

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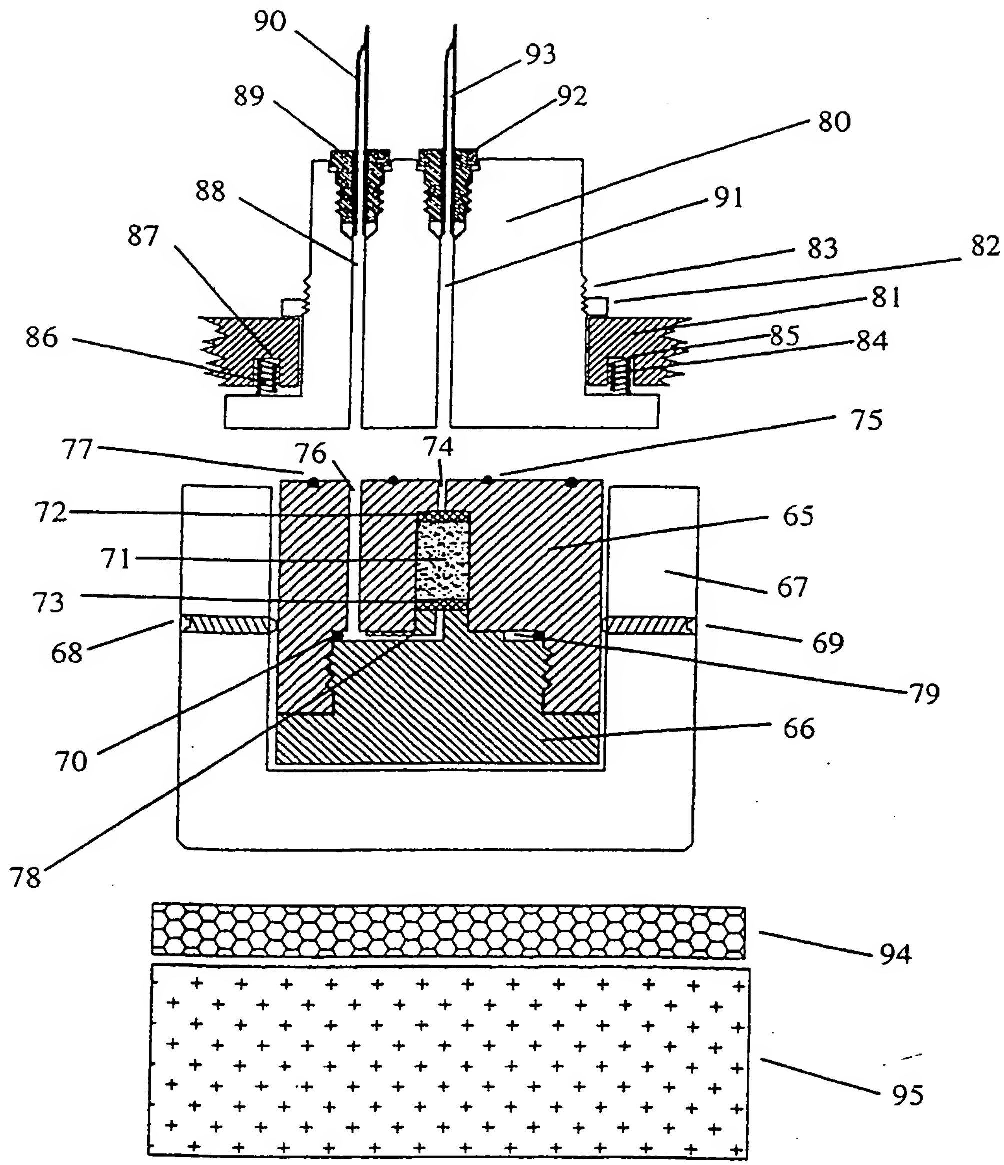
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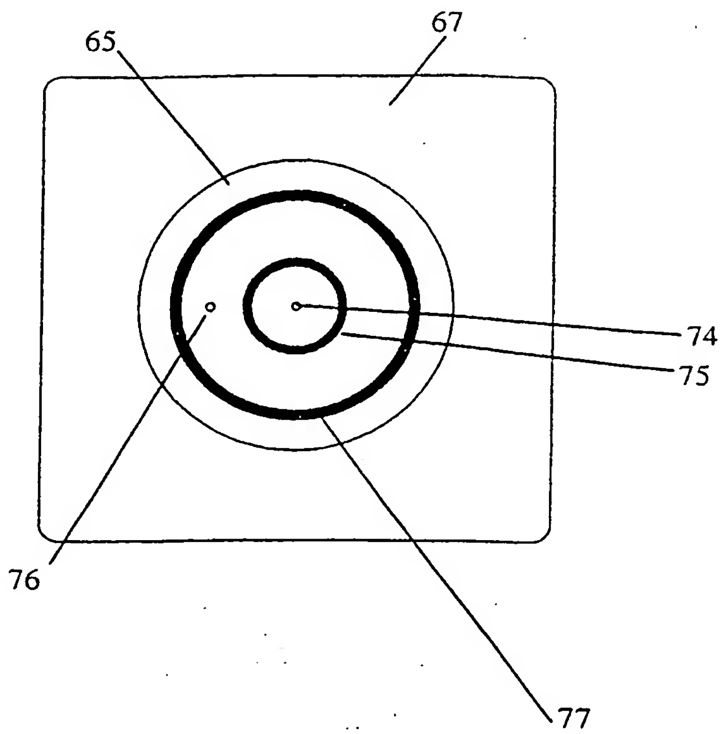
Figure 15



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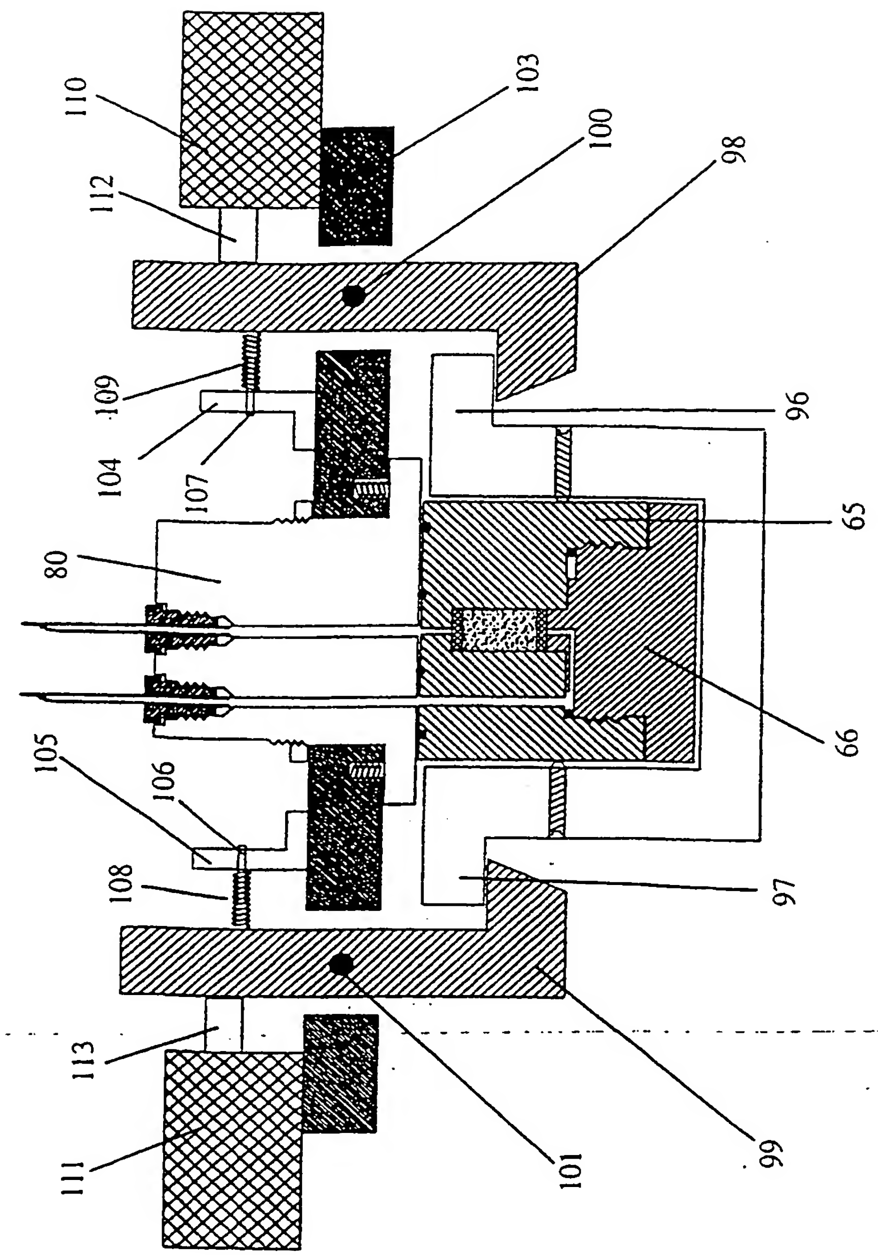
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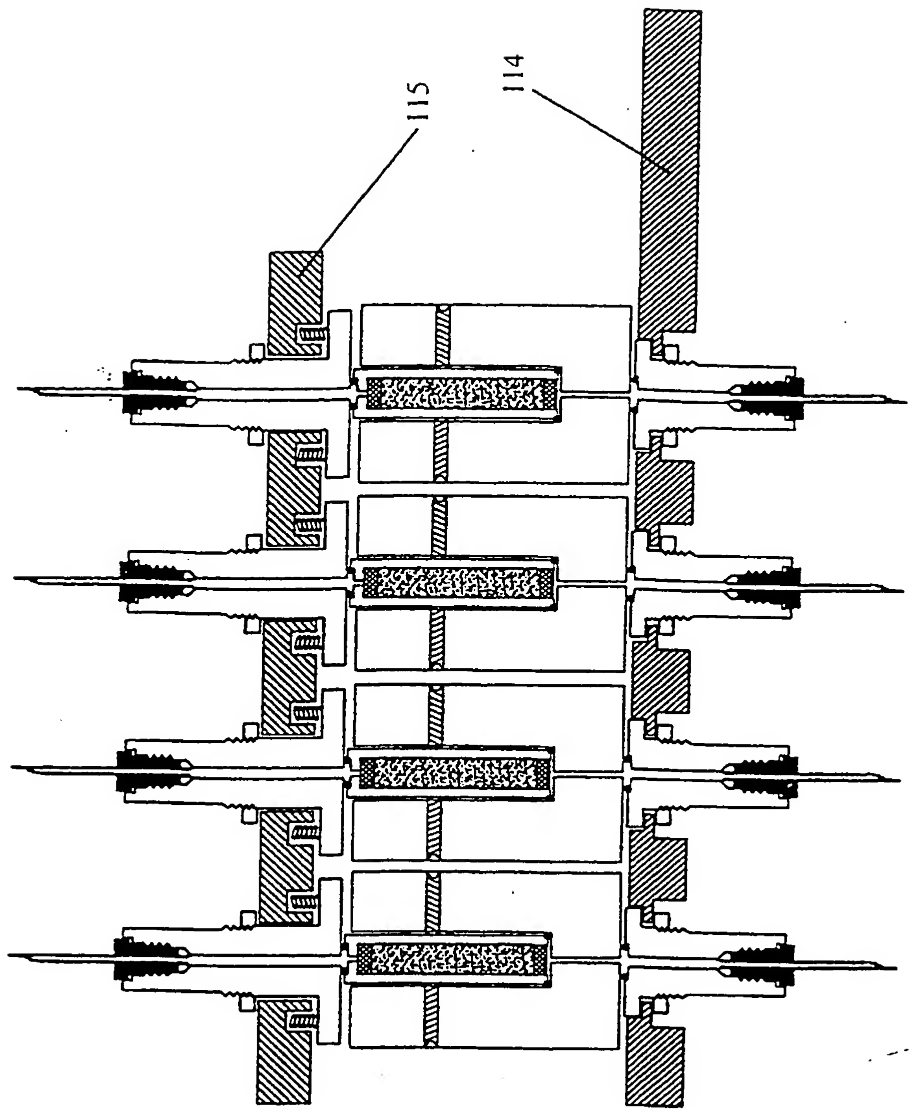
Figure 17



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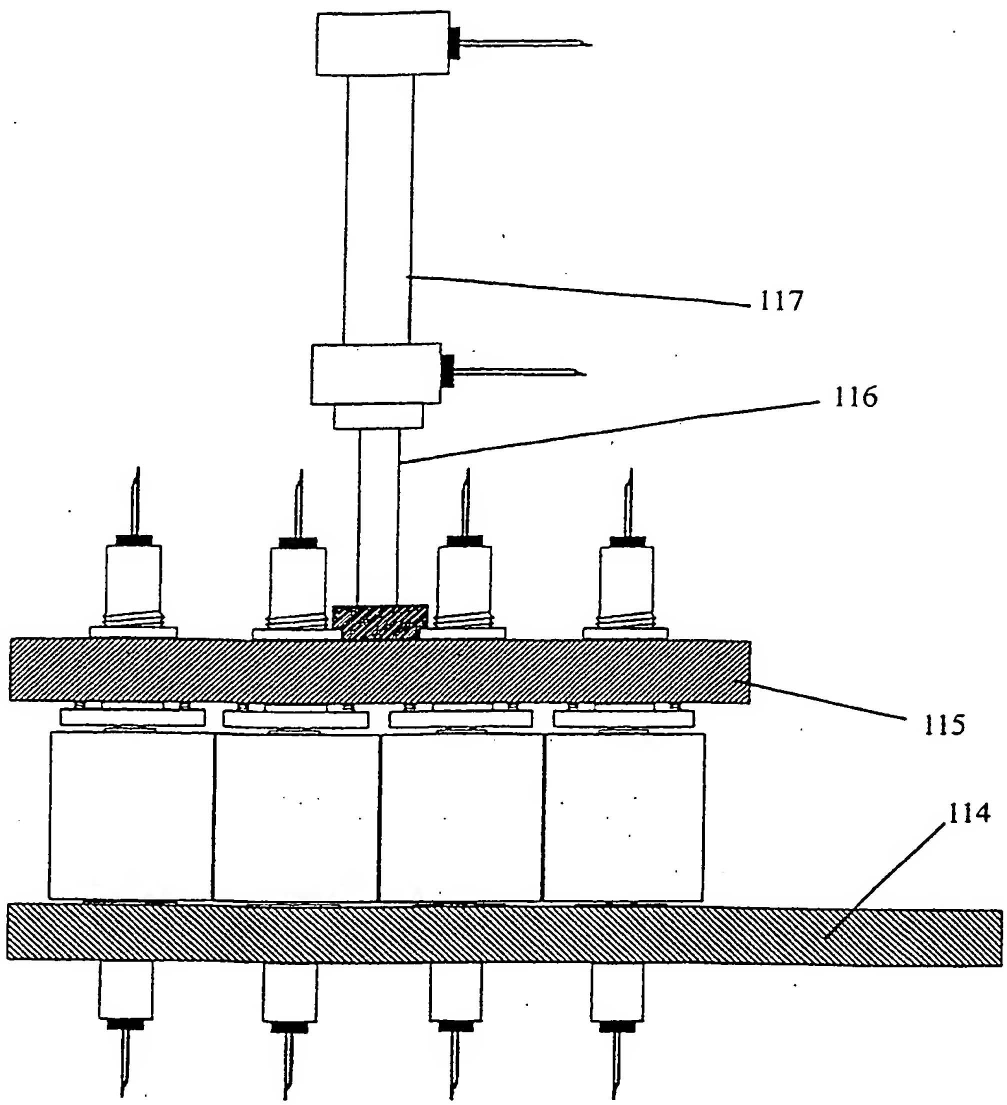

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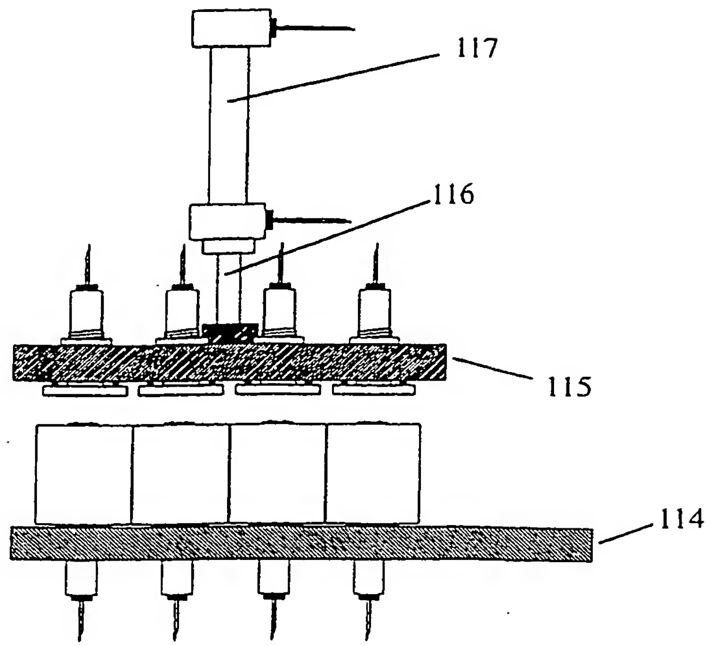

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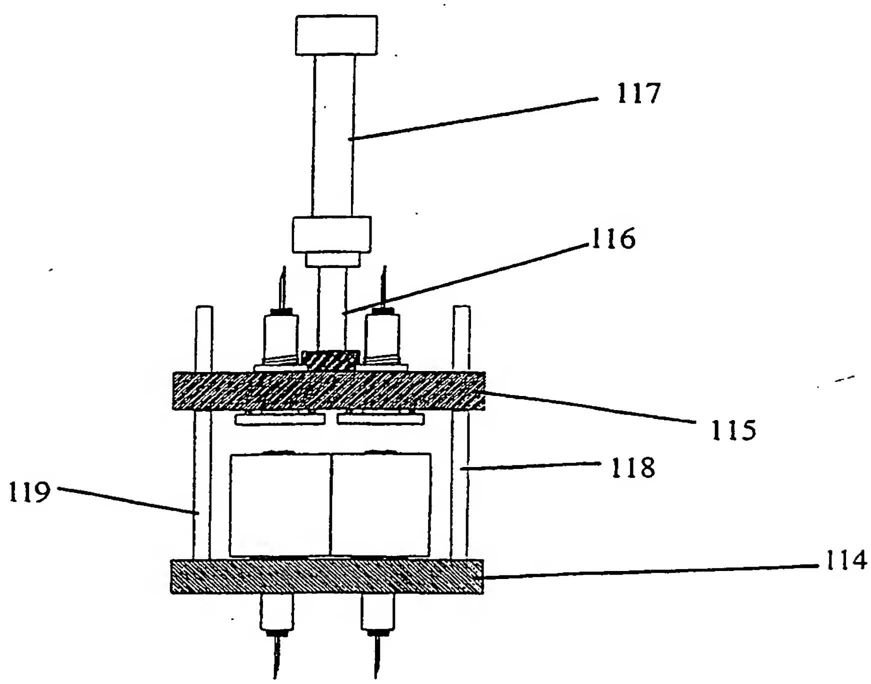

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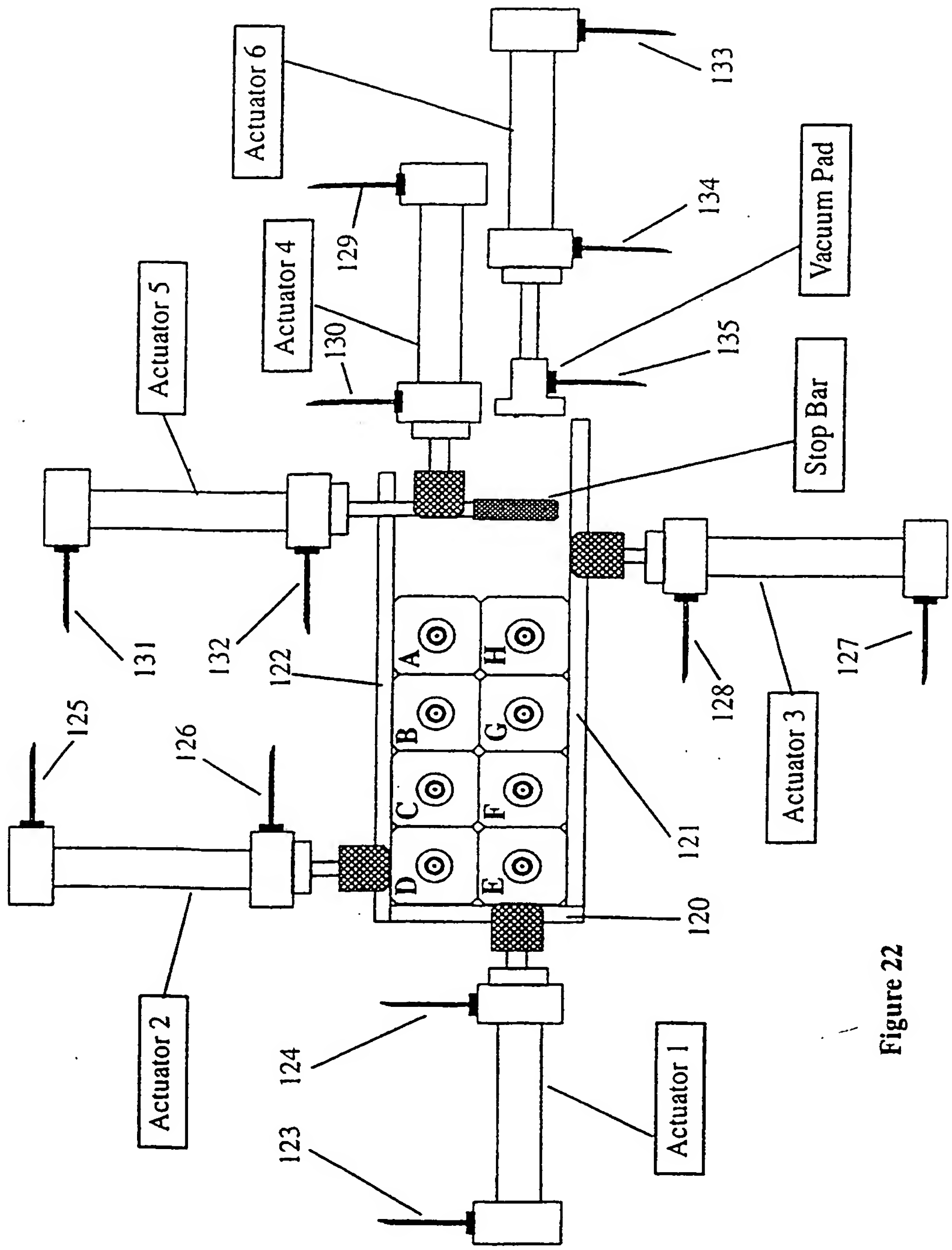
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Actuator 5
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Actuator 2
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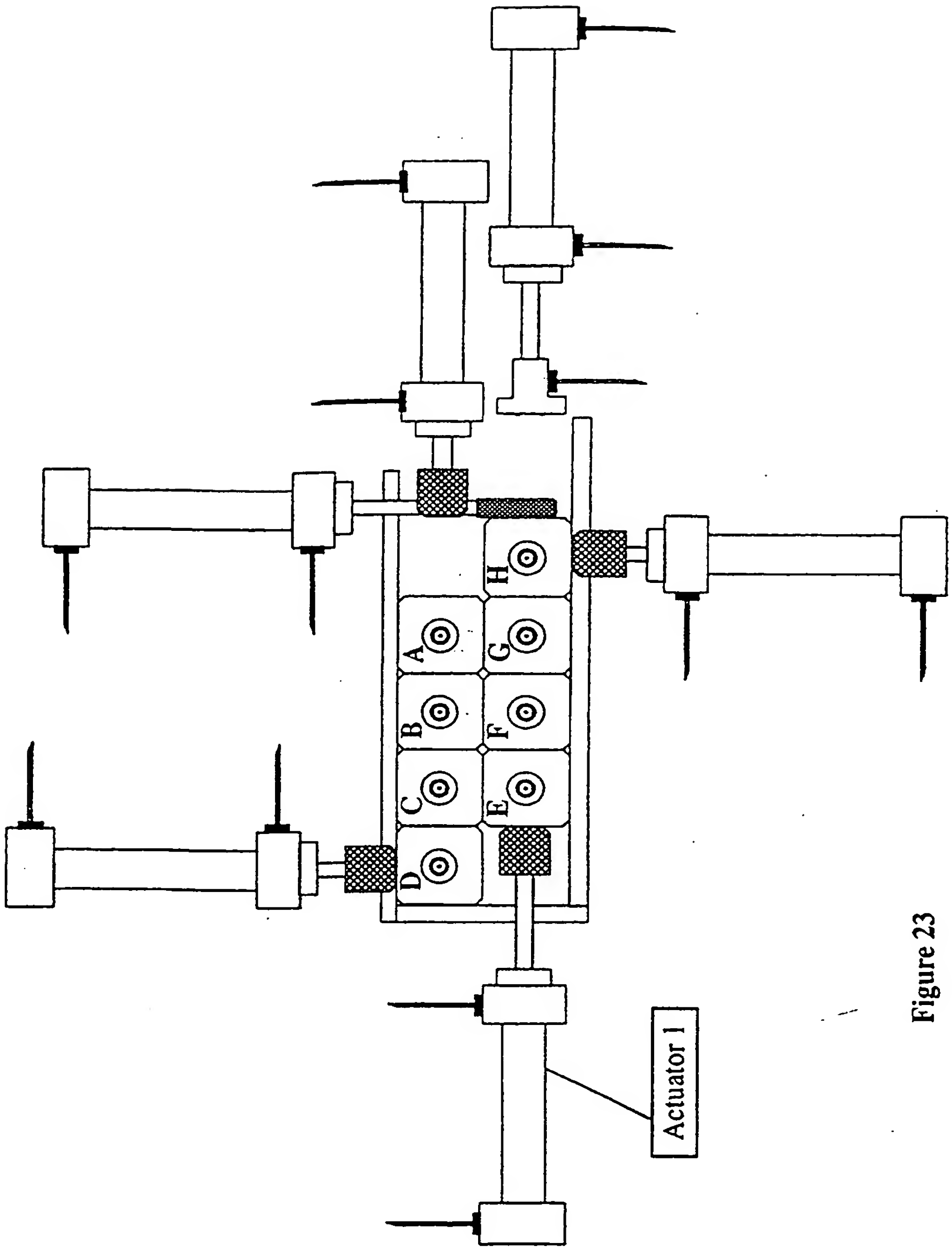
Actuator 1

Figure 22



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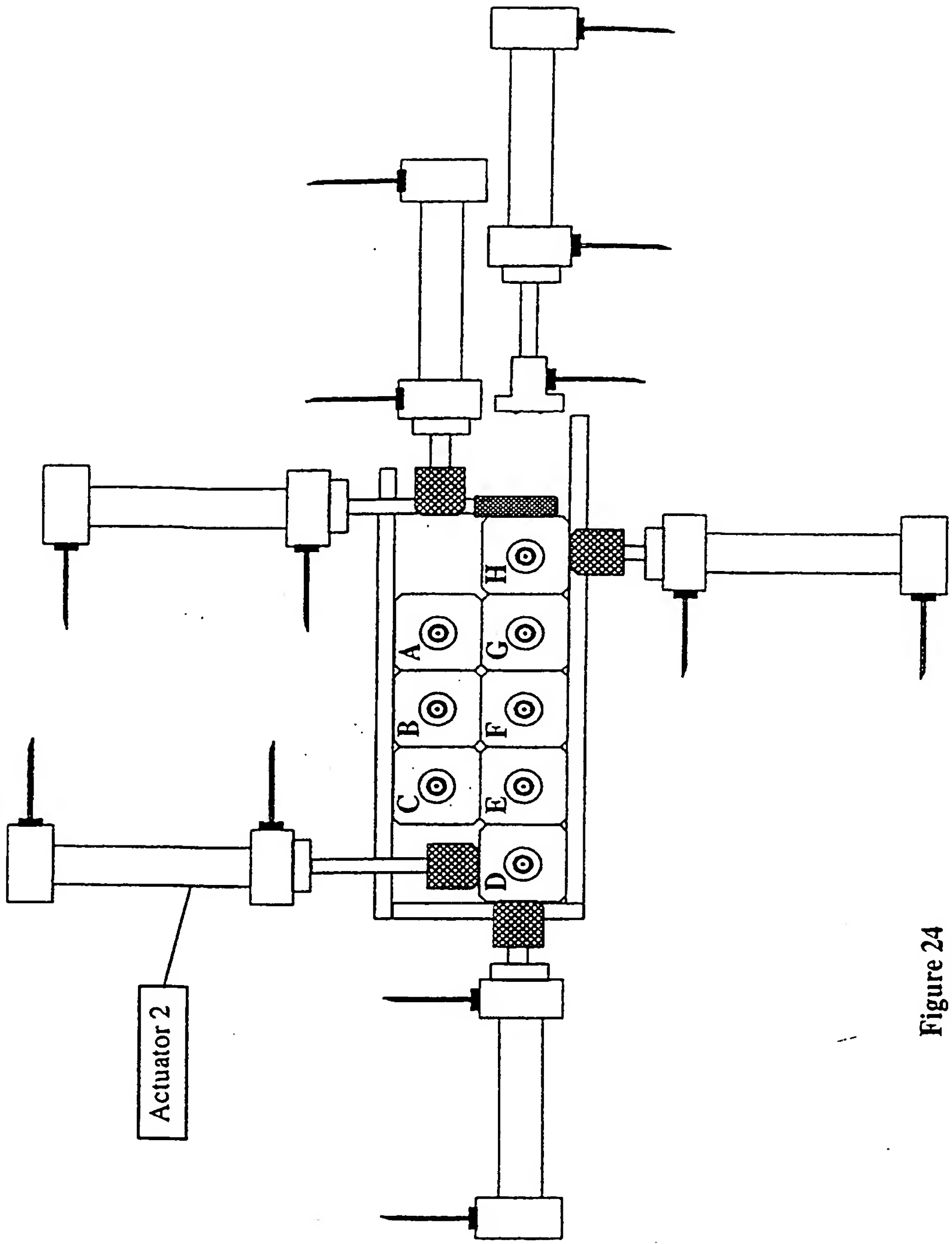

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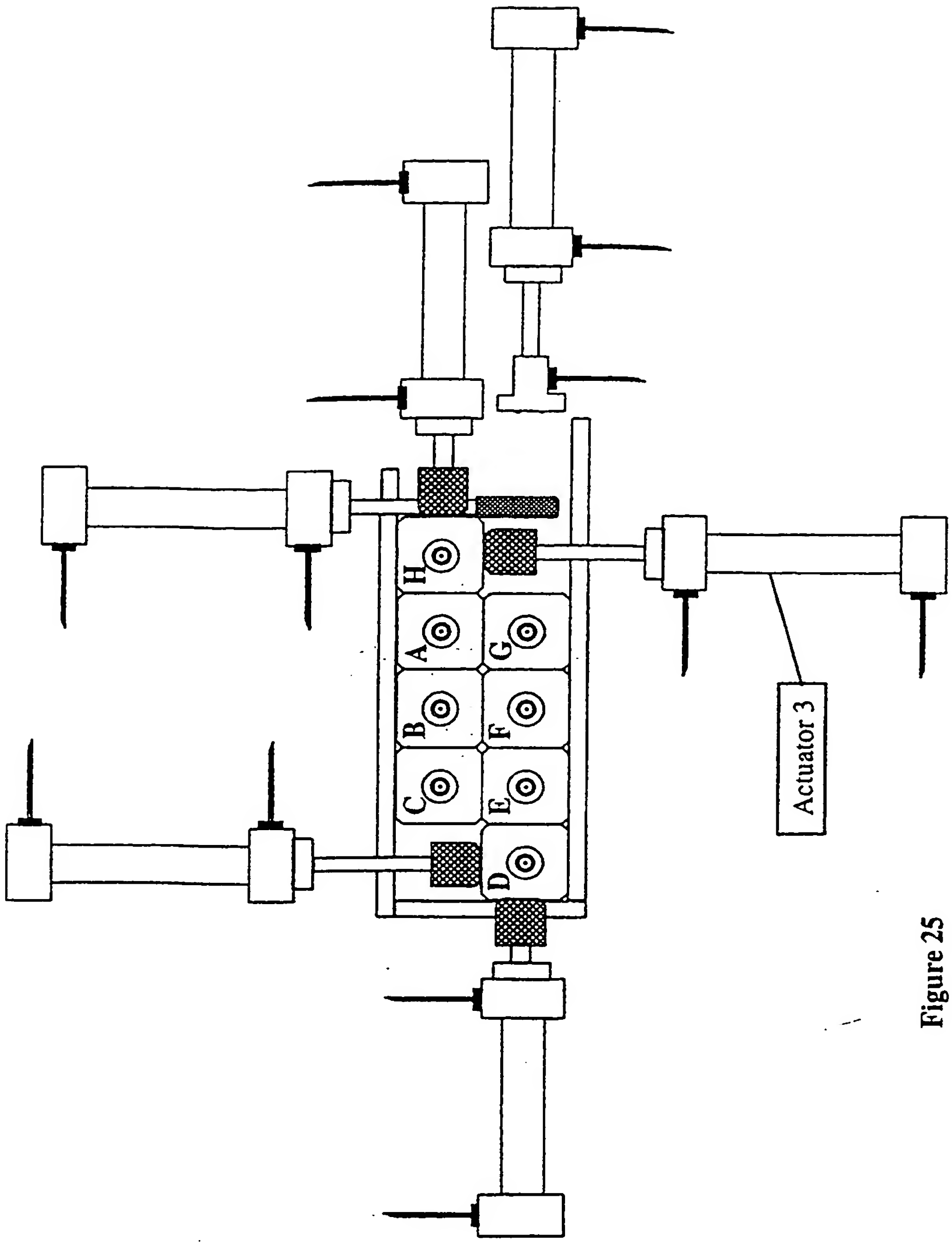

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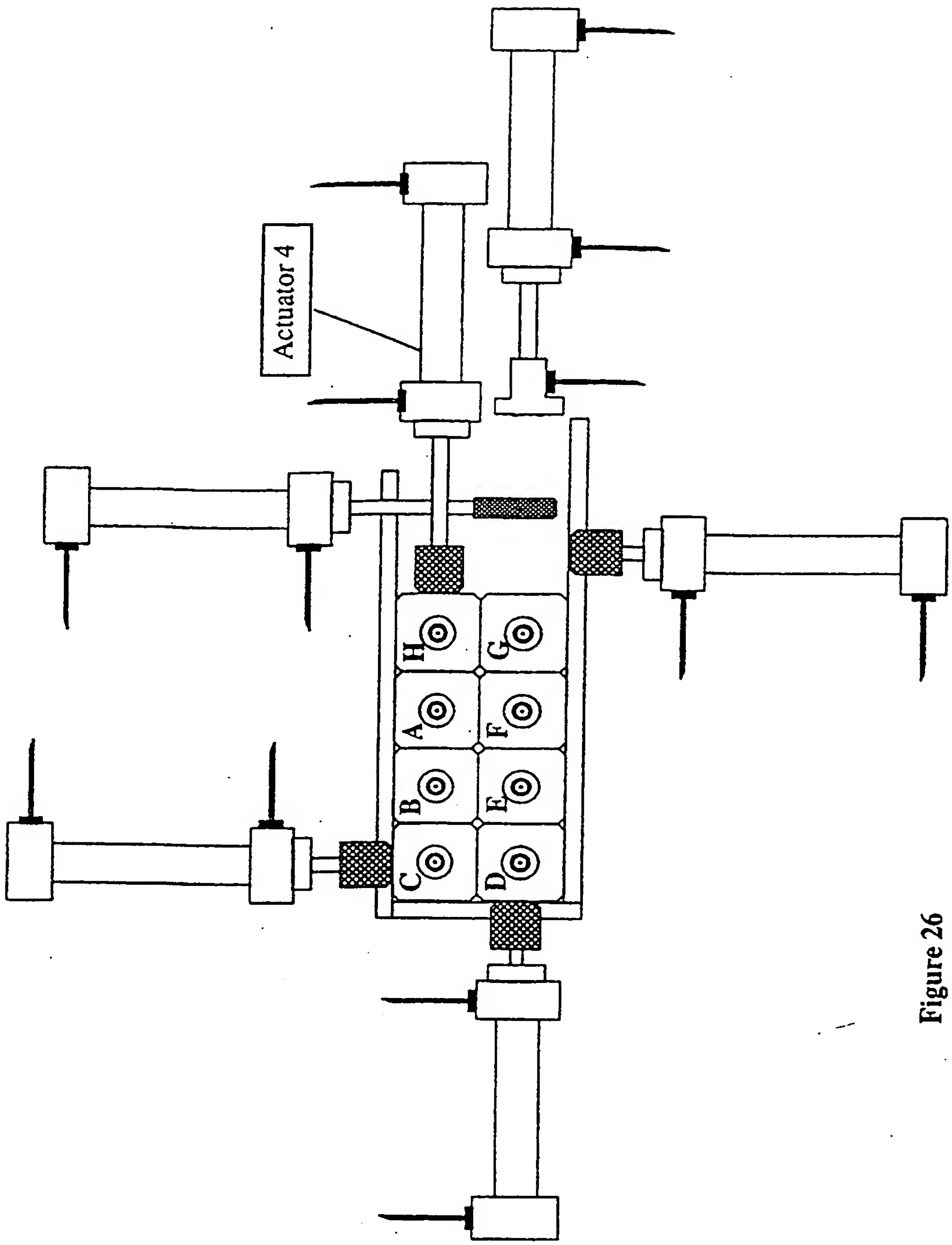

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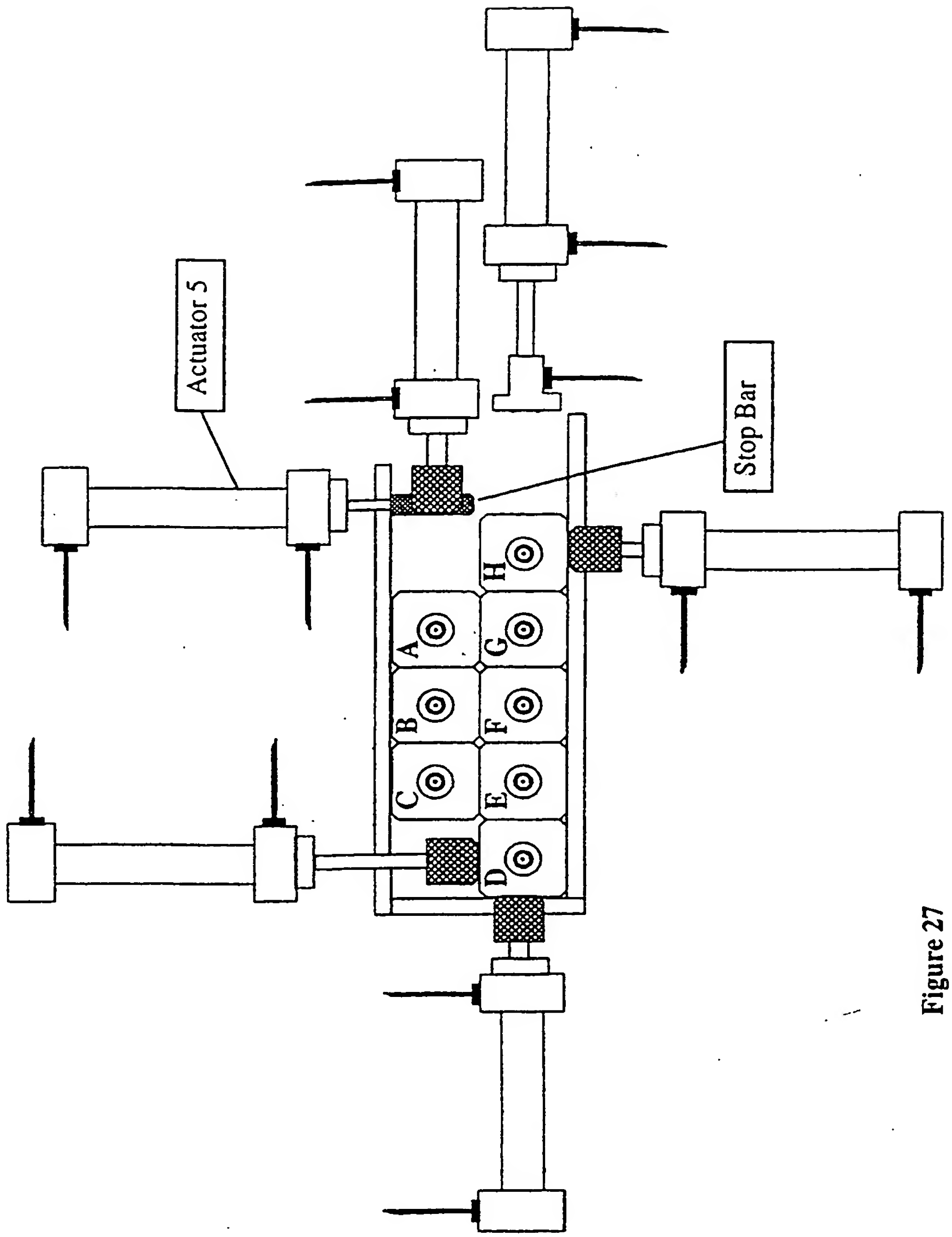

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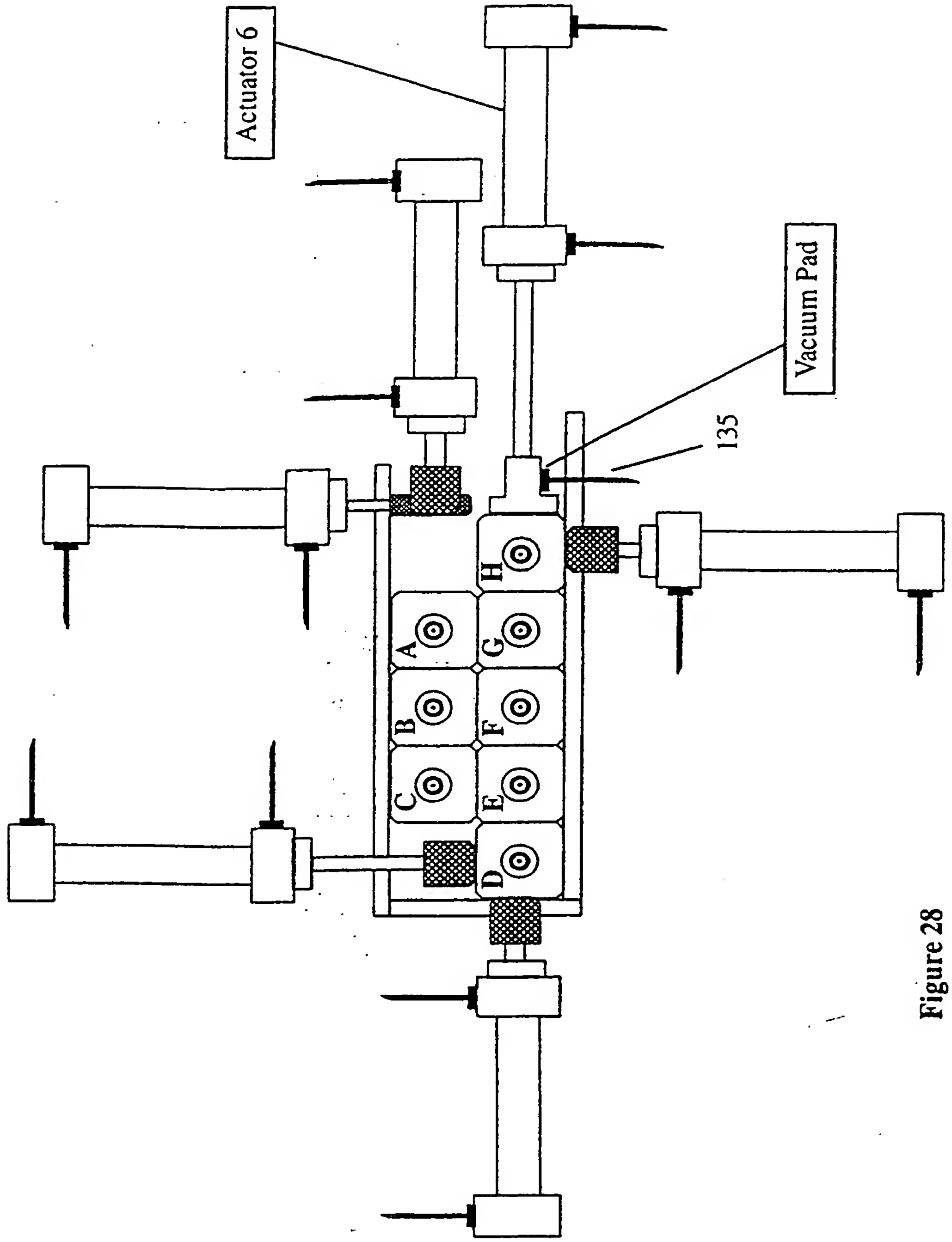

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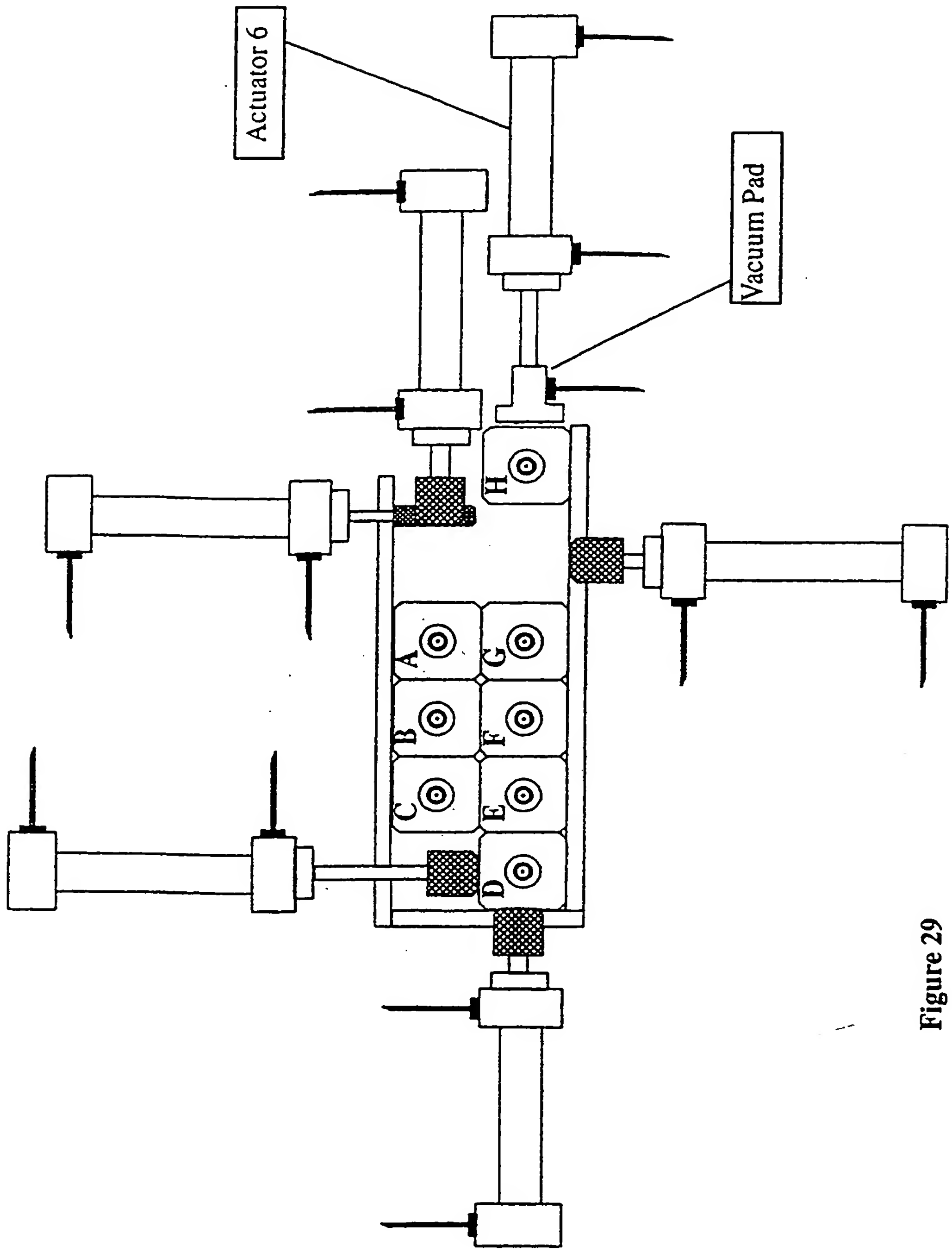

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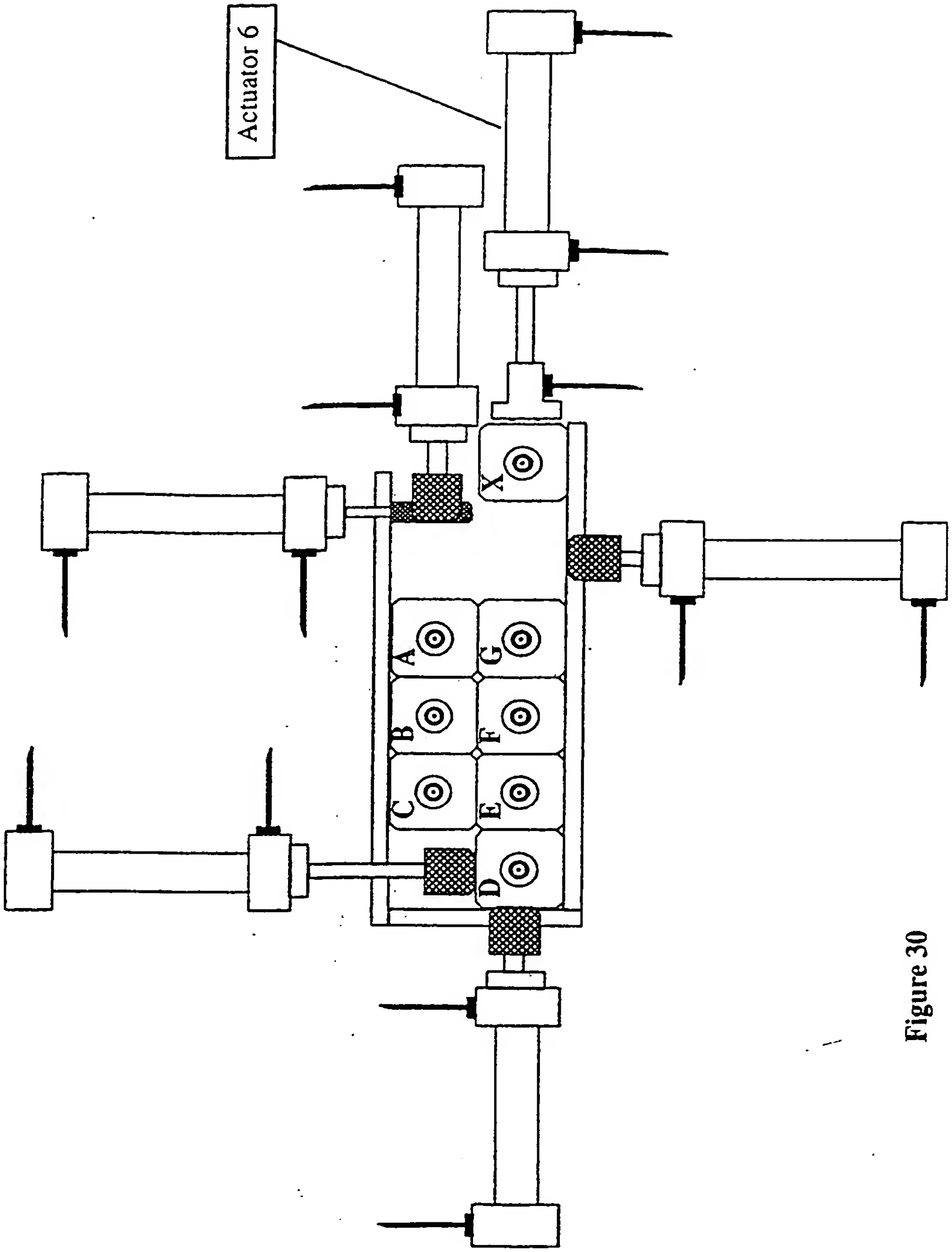
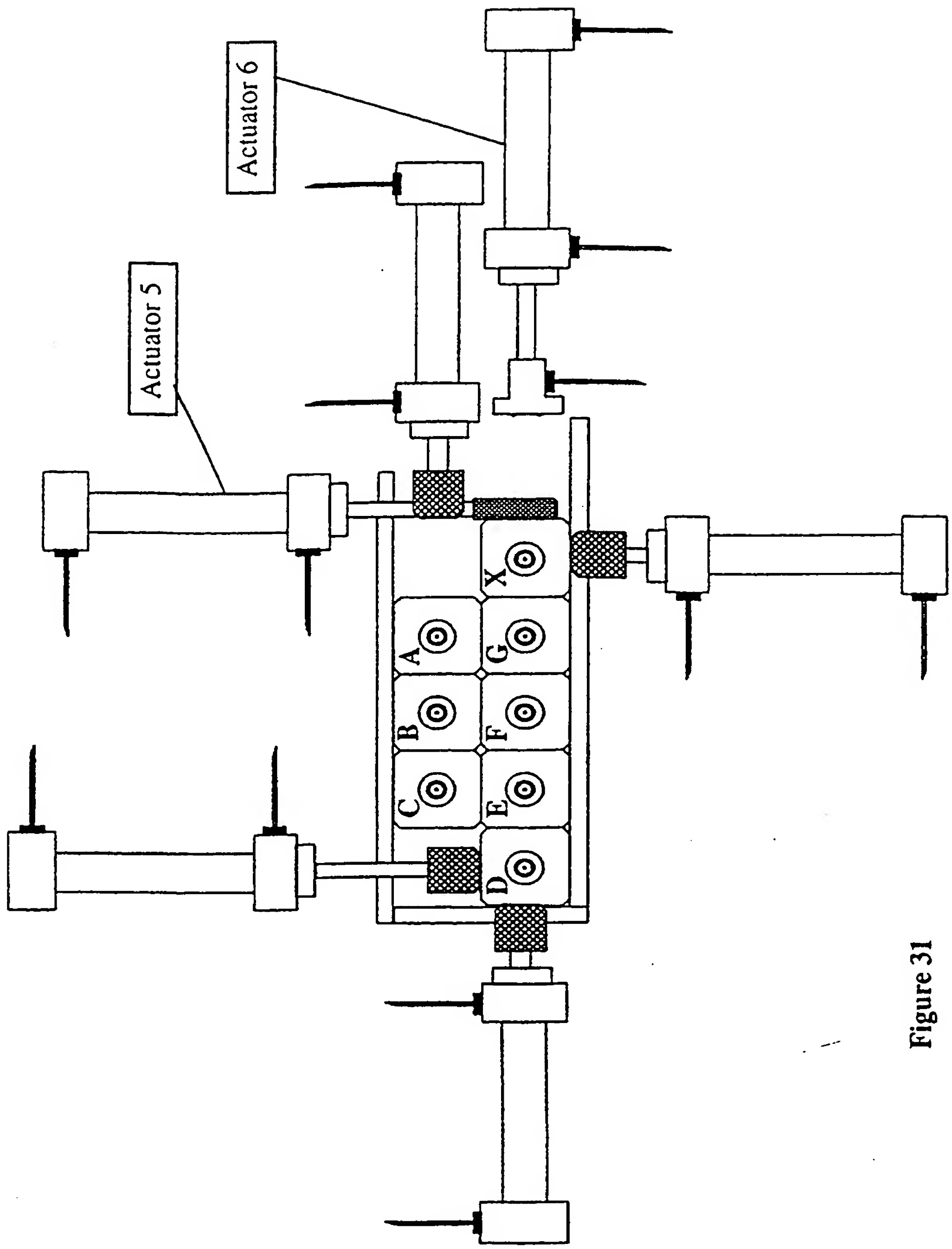


Figure 30



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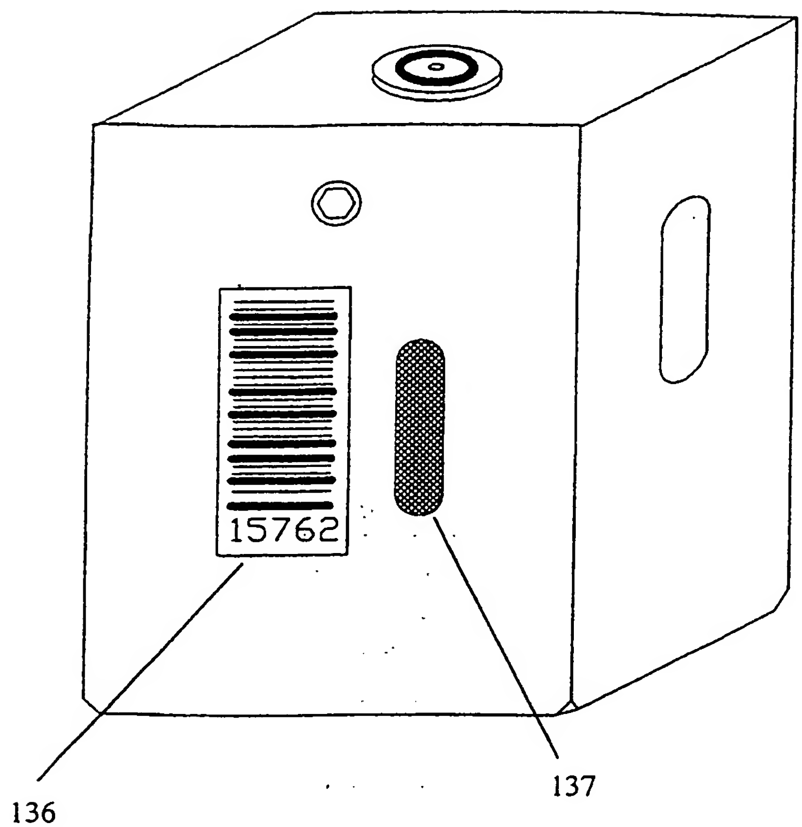


Figure 32

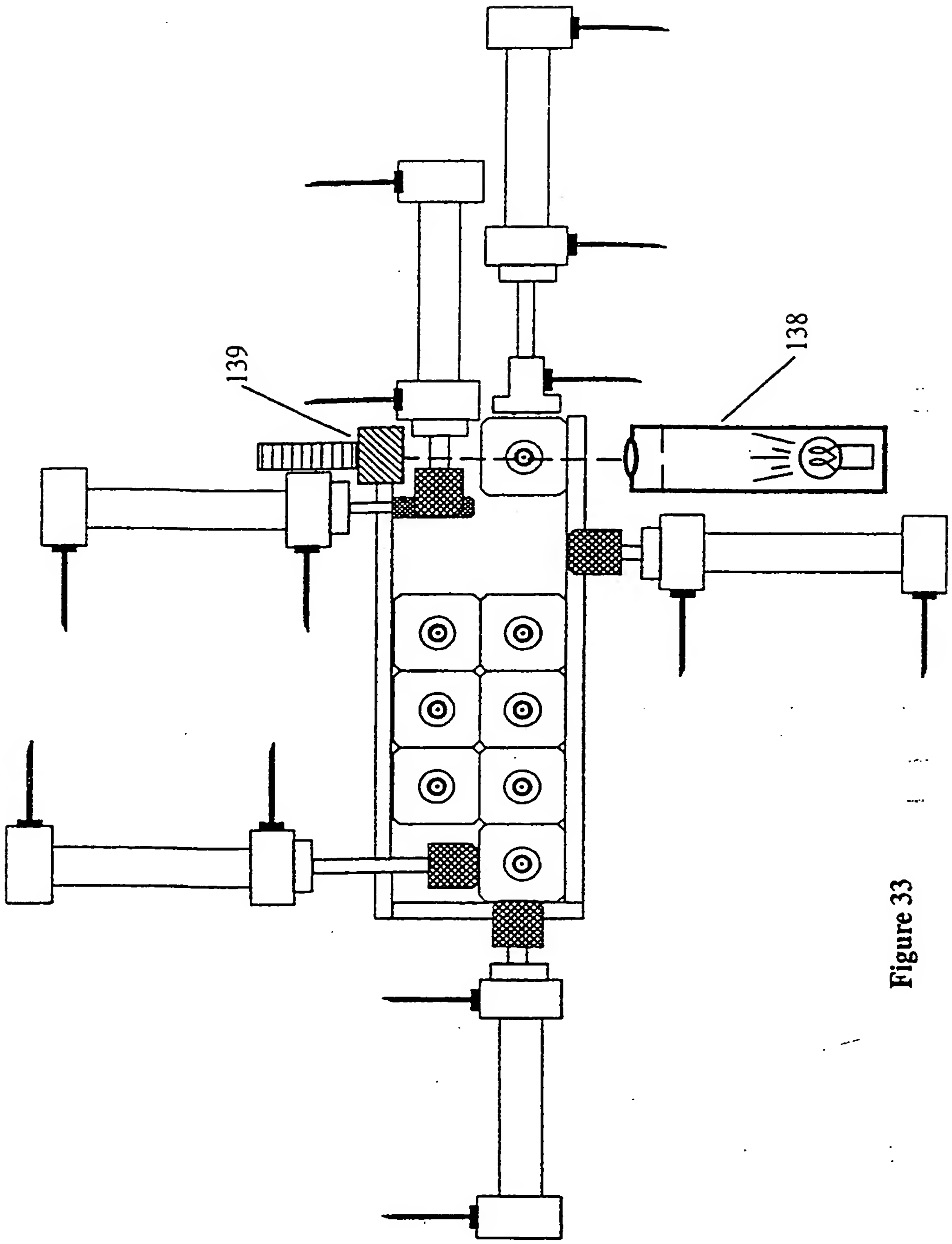
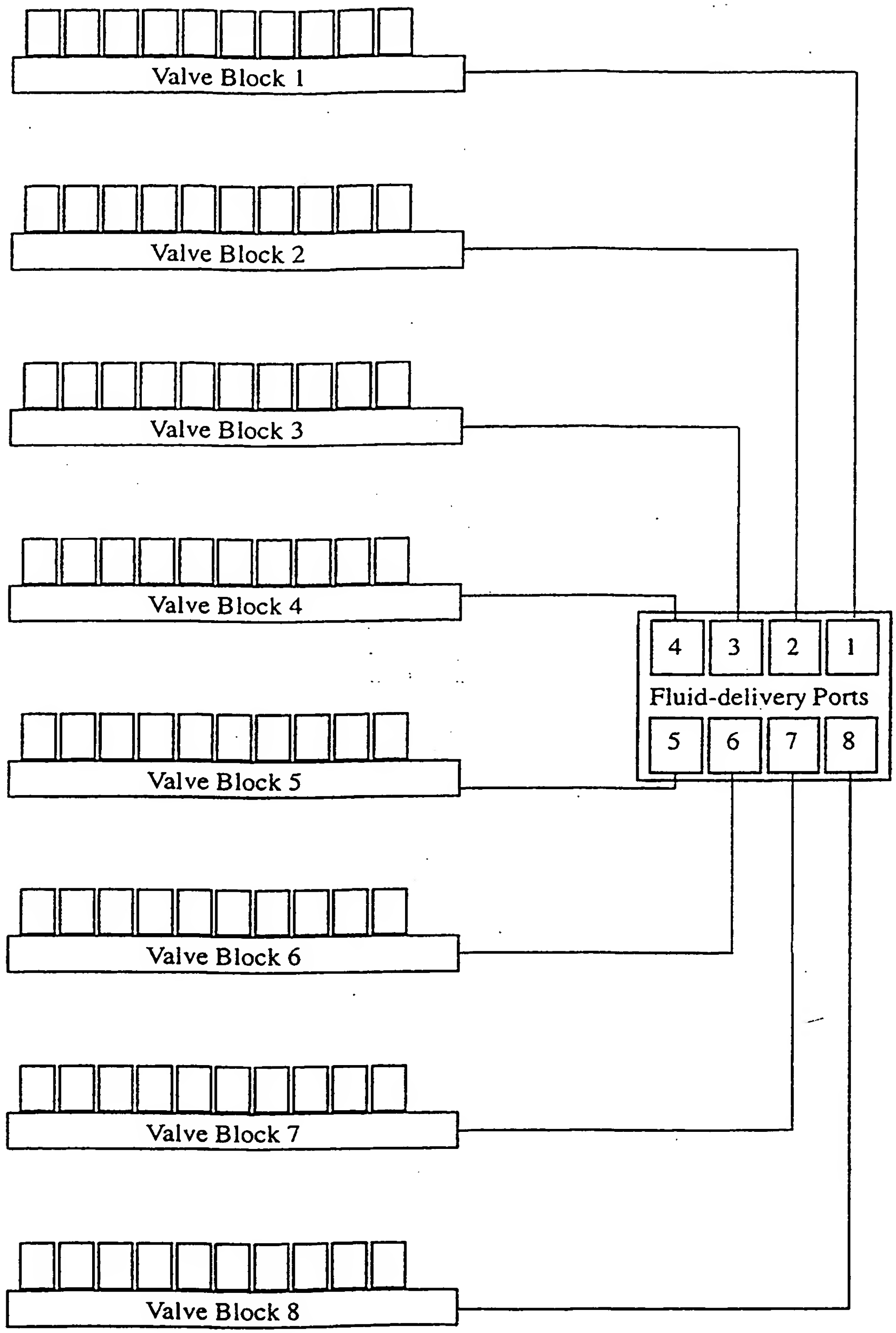


Figure 33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Fluid-delivery Ports
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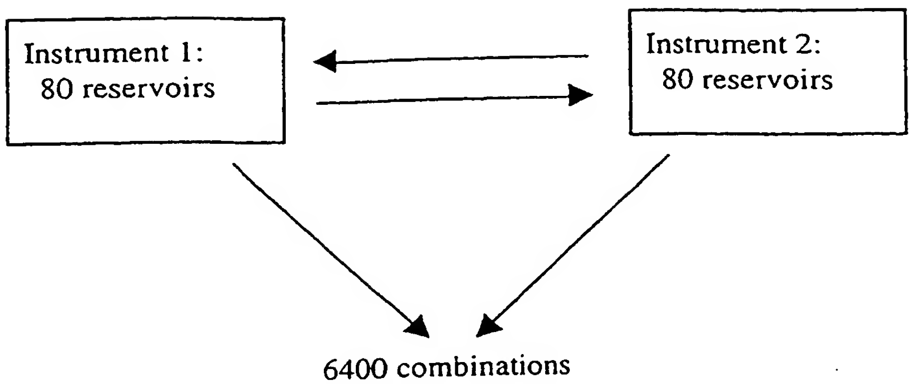
Figure 34



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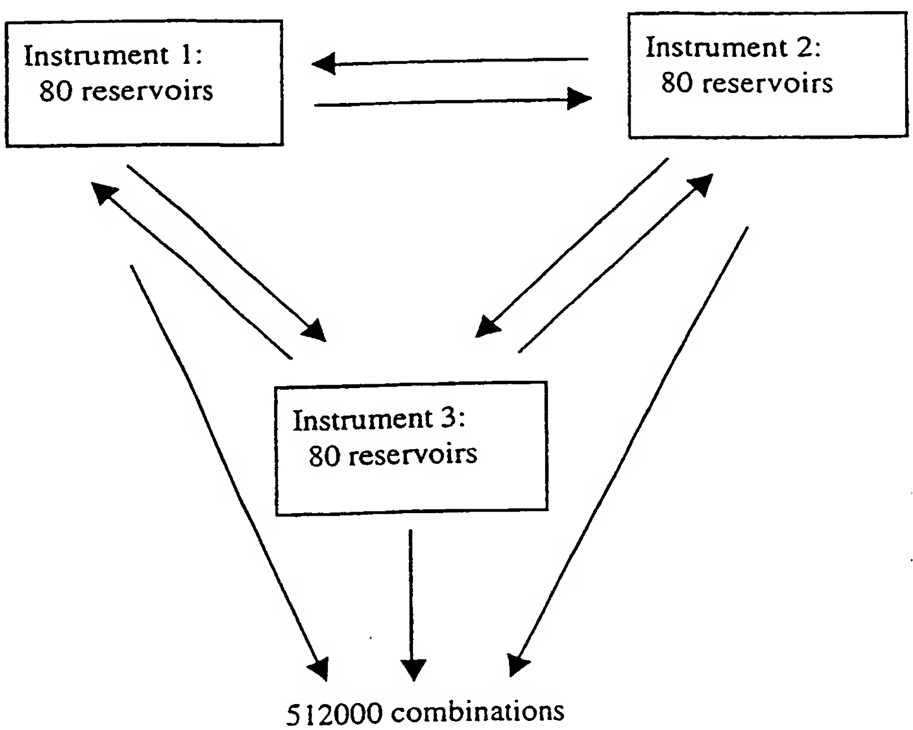

Figure 36